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SECTION ONE

# INTRODUCTORY TOPICS

# 1

# Heterosis : An Introduction

The term *heterosis*, as is now widely used, refers to the phenomenon in which the $F_1$ population obtained by the crossing of the two genetically dissimilar gametes or individuals shows increased or decreased vigour over the better parent or over the mid-parental value. Shull referred to this phenomenon as the stimulous of heterozygosis and in his words it has been the "interpretation of increased vigour, size, fruitfulness, speed of development, resistance to diseases and to insect pests or to the climatic rigours of any kind manifested by the outbreeding organisms as compared with the corresponding inbreds as a specific result of the unlikeliness in the constitution of the uniting parental gametes." It is now widely recognised that this phenomenon is the result of the action and interaction of the unlike gametes in the heterozygote (Aa) and the heterosis is only the better or worse than expected manifestation of this biological behaviour of the hybrid.

## HETEROSIS AND HETEROBELTIOSIS

As described earlier, *heterosis* is the increased or decreased vigour of $F_1$ over its better parent or the mid-parental value. In plant breeding programmes, conventionally *heterosis* is referred to denote the expression of increased vigour of the hybrid over the better parent. But since *heterosis* is also expressed over the mid-parental value, it needs some distinction. Recently a new word *heterobeltiosis* has been proposed (Bitzer, *et al.* 1968; Fonseca and Patterson, 1968) to describe the improvement of the heterozygote in relation to better parent of the cross and now this term is precisely being used to connote the expression of heterosis over the better parent.

### General aspects of the expression of heterosis

The general features of the phenomenon of heterosis are as follows :

(i) Heterosis is a widely occuring biological phenomenon in both, the plants and animal species. In plants, it has been reported to occur more frequently in a number of naturally cross pollinated crop species, as compared in the self pollinated ones.

(ii) It is a built-in evolutionary mechanism in an organism which favours the better survival of the heterozygotes in nature. It permits and protects a

number of recessive genes from being eliminated under natural conditions in the outbreeding organisms.

(iii) In a particular crop or animal species, it is usually observed that not all the hybrids show heterosis but is exhibited by only a few and the specific ones.

(iv) Heterotic crosses usually show increase in size, vigour, seed produeing capability, usually better resistance to insect pests or diseases, increased metabolic activity and better stability and thus ultimately result in the better performance of the hybrids than the parents (Fig I.1). Usually, these hybrids show better fitness and breeding value as compared to the parents from which they are made.

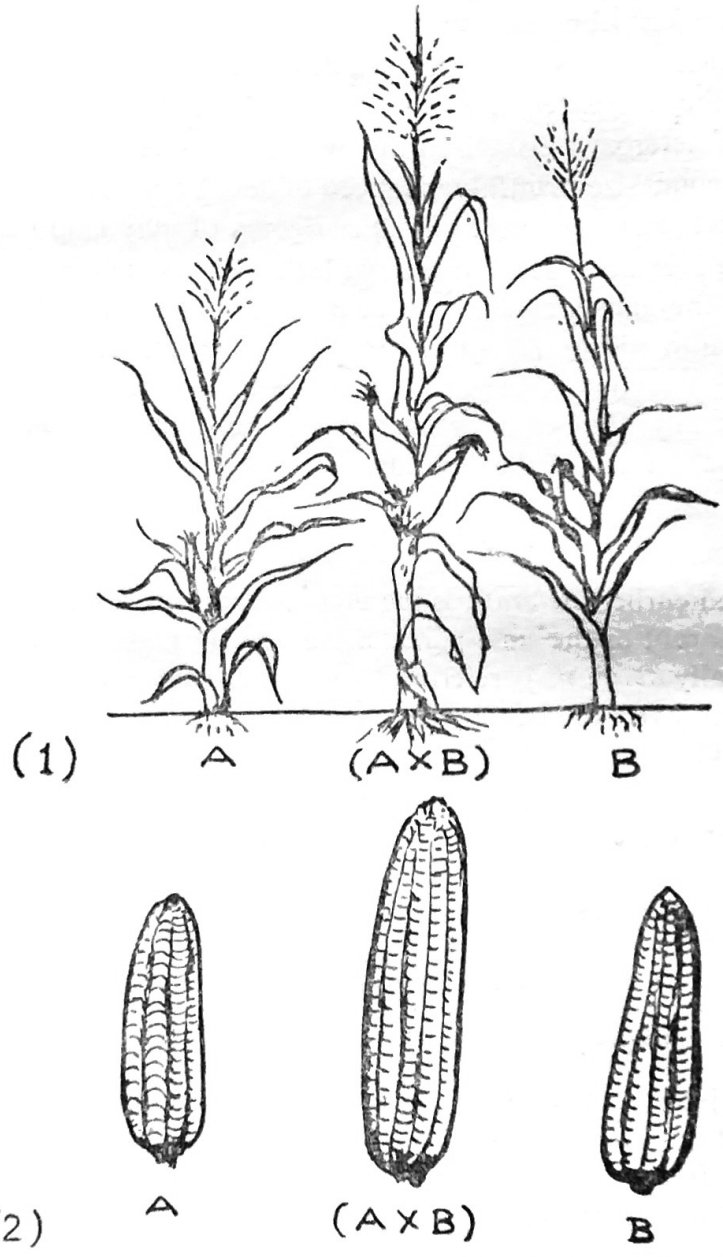


*Fig. 1.1* : Expression of heterosis in maize (1) Plants of line A, hybrid (AxB) and line B. (2) Ears of line A, hybrid (AxB) and line B respectively.

(v) Elaborate experiments on heterosis have shown that it is a genetically governed phenomenon.

(vi) This phenomenon is confined only to the specific $F_1$ or the first hybrid generation and considerably deminishes in the $F_2$ and the later segregating generations. This reduction in the performance in later generations is because of the breakdown of specific gene combination building up the expression of heterosis.

(vii) It has been a common observation that the expression of heterosis is usually more in the hybrids obtained from the genetically diverse or unrelated lines than those from the genetically related lines. The expression of heterosis in a crop species shows that some degree of genetic diversity, heterozygosity and or dominance exists in the parents with respect to the character or characters in which this phenomenon has been expressed.

(viii) In terms of combining ability for quantitative characters, the expression of heterosis is highly associated with the specific combining ability of a cross.

(ix) Heterosis is usually unfixable from generation to generation as the heterozygote advantage is confined to $F_1$ population only. But some natural contrivances which favour the existence of heterozygotes or those which preserve the heterozygote condition from generation to generation could fix it. Some such mechanisms in nature are the existence of homozygous lethal factors, balanced heterozygotes, apomixis or the use of vegetative reproduction.

(x) In the cultivated crop plants, this phenomenon has been adequately reported in crops like maize, sorghum, bajra, brinjal, brassica, onion, tobacco, tomato, wheat, barley, rice, castor, cotton, sunflower, sugarbeet, raddish, carrot, cucumber, squashes, grasses, legumes, etc. and has been commercially utilized in crops like, maize, onion, bajra, sorghum, sugarbeet, tomato, etc.

In the case of the insects, birds and other animals, it has been frequently observed and reported in silkworm, fruit fly, doves, poultry, turkey, swine, sheep, cattle, etc. and has been commercially exploited in the case of poultry, swine, etc.

**Types of heterosis**

Depending upon the nature of the origin, adaptability, reproducibility and non-reproducibility, heterosis could be classified into two classes : (i) Euheterosis (True heterosis), and (ii) Pseudoheterosis (Luxuriance).

Euheterosis could further be divided into : (a) Mutational euheterosis, and (b) Balanced euheterosis; depending upon the nature and origin of the genes causing heterosis in a biological system (Dobzhansky, 1952). A brief description of these types of heterosis is as follows :

*Mutational Euheterosis*

This is perhaps the simplest type of euheterosis and results from the sheltering

or overshadowing the deleterious, unfavourable often lethal, recessive mutants by their adaptively superior dominant alleles in population of sexually reproducing cross fertilizing organisms (Dobzhansky, 1952). Most of the mutations which occur in natural conditions are genetically recessive and adaptationally unfavourable. The phenomenon of mutational euheterosis shelters the deleterious recessive mutants and for them works as the protective device against the deleterious mutation pressure. For the newly evolved mutants, this would perhaps be necessary. Because a large number of such mutations which occurs under natural conditions are deleterious to organic evolution and are easily liable to be wiped out by natural selection. Mutational heterosis shelters and protects them from their quick elimination. In essence, it is the result of the natural consequences of the phenomenon of dominance.

*Balanced euheterosis*

Balanced euheterosis is a type of true heterosis which arises out of a balanced, specifically adapted gene combination or from the occurrence of rather special type of adapted mutations which confer on the heterozygote a better adaptive value or a higher agricultural usefulness than is usually found in the corresponding homozygotes. There are a number of recessive genes in nature which are comparatively less favourable for their natural adaptation than their dominant counterparts and are consequently liable to be lost. This phenomenon of specific gene recombinations functions as a built-in evolutionary mechanism which maintains the multiplicity of the adapted recessive genes in a population, provides additional vigour to the heterozygote and saves it from being lost. This makes the cross pollinating species virtually and potentially self improving ones under natural conditions. In essence, the balanced euheterosis is a culmination of the phenomenon of over-dominance.

*Luxuriance*

Luxuriance is basically a phenomenon by which the crossing of the two parental forms brings in an accidental, excessive, usually unadaptable and an unbalanced expression of an attribute. Some such examples are seen in the excessive vegetative growth of foliage, stem, flower buds, etc. and is for more encountered in the case of the domesticated than the wild species. This is usually thought to be resulting from the complimentary action of the genes found in the two parental species crossed. Though this phenomenon resembles like heterosis and has been noticed in many domesticated species but it has not been found to be agronomically and economically feasible to utilize. This is primarily because of the reasons of adaptability under the natural conditions of crop production. Usually there is no indication whatsoever or any conclusive proof that the luxuriant hybrids would also simultaneously prove adaptively superior in comparison or in competition with the parental forms in the natural habitat of the latter.

**Measurement of heterosis**

In a particular cross, heterosis is usually measured in terms of two parameters,

namely (i) heterosis over the mid-parental (MP) value, and (ii) heterosis over the better parent (BP). In plant breeding programmes, however, it is also estimated in terms of heterosis over the check variety. For commercial exploitation, this value is, perhaps, the most important one. The procedure for the estimation of these values are as follows :

(i) Heterosis over the mid-parental value (Relative heterosis)

$$= \frac{F_1 - MP}{MP} \times 100$$

where $F_1$ and MP are the average performances of the $F_1$ and the mid parental values respectively.

(ii) Heterosis over the better parent (Heterobeltiosis)

$$= \frac{F_1 - BP}{BP} \times 100$$

where BP is the average performance of the better parent.

(iii) Heterosis over the check parent (Standard heterosis)

$$= \frac{F_1 - \text{Check variety}}{\text{Check variety}} \times 100$$

where check variety denotes its average performance.

**Calculation of heterosis from the gene effects**

Based on the estimates of the gene effects obtained from the six components model of the analysis of quantitative gene action, *i.e.*, additive (a), dominance (d) and its various interaction components like (a × a) (a × d) and (d × d), Jinks and Jones (1958) have suggested the following formula for the calculation of heterobeltiosis at the genotypic level.

Heterobeltiosis = (dominance — additive × additive) —
(additive + 1/2 additive × dominance)

Symbolically, it could be expressed as :

$$F_1 - BP = (d - a \times a) - (a + 1/2\ a \times d)$$

**Value of heterosis**

In terms of increasing the productivity of crop plants in general and cross pollinated crops in particular, the relative power and the potency of heterosis breeding is enormous. It has already become a thoroughfare in the breeding of cross pollinated crops like maize, bajra, onion, sugarbeet, etc. and is now also being utilized in increasing the productivity of the predominantly self-pollinated crops like sorghum, wheat, etc. As could be visualized in Table 1.1, the use of hybrids have rapidly replaced the use of open pollinated maize varieties in the U.S.A.

TABLE 1.1

**Use of hybrid maize in the U.S.A.**

| *Year* | *Percentage of total maize area under hybrids* |
|---|---|
| 1933 | 1 |
| 1940 | 50 |
| 1944 | 80 |
| 1955 | 98 |
| 1970 | 99 |

A recent survey has indicated that the use of the hybrids has cut the relative per unit cost of production in the U.S.A. The cost of production per bushel in case of open pollinated varieties was found to be 1.10 dollars while that of hybrids at that level of production was 0.90 dollars (Smith, 1966).

In India, the use of hybrid varieties in case of sorghum, bajra and maize is replacing the area under the open pollinated varieties. At least a conservative estimate of hybrid maize has shown that acreage under hybrid maize has been increasing from less than one lakh in 1964 to more than seven lakhs in 1967. Though the accurate estimates are not available for sorghum and bajra, the area under the hybrids in these crops is also fast increasing. The use of hybrids now appears to be one of the main factors in increasing the production of these crops in India.

# 2

# History of Heterosis Breeding

A glimpse of the major milestones in the development of the heterosis concept and its usefulness as a breeding tool can be discussed in three different stages :

(a) Early ideas on inbreeding, outbreeding and the expression heterosis (*i.e.* upto 1900),

(b) The contributions of Shull and East (1905-12), and

(c) The later developments in the heterosis breeding methodology.

A brief description of these phases has been given as follows :

### (a) Early ideas on inbreeding, outbreeding and the expression of heterosis

Inbreeding and outbreeding are the two systems of matings involved in the sexual reproduction of the plants as well as the animal species. Each one of these species biologically adjusts to its favoured system of mating in its biosphere and naturally discards the unwanted other. Since the dawn of civilization and in the recorded history, there have been quite a number of informations and stories on the practice of inbreeding and the outbreeding, their ills as well as also the favourable effects. They have been recorded more in human beings and have been recorded rarely in animals and plants. Only since the last 200 years, when a concentrated and vested interest started on the understanding of the plants and their breeding for food and fibres, etc., the effect of inbreeding, outbreeding and the presence of heterosis has been systematically recorded and reported.

The recorded history in the old testaments, religious history books amply reveals that the inbreeding, though rather rare in human beings and animals, was considered to be desirable and was usually practised for saving the purity of the blood in the cases of ruling monarchs, kings, queens as also some times for the reasons of saving the property from being lost by the family. The ancestry of Christian and Greek Gods and Goddesses is filled with such stories of inbreeding (Zirkle, 1952). It was, however, also invariably practised for the breeding of noble and rare horses. Inbreeding was more or less a compulsion in the life of the islands isolated by the vast deep seas, etc. But most of the conventional practices of inbreeding diminished considerably with the realization of their ill effects, *i.e.* with the reduction of the vigour, size and

the production of sterile individuals and ultimately for the reasons of the psychological detractions in human and animal species. The Hindu religion had been advocative of the distant marriages for the production of better progenies and the whole system has been based on the union of rather distant individuals. Even to-day, it forbids the close inbreedings by religious beliefs and customs.

For the first time in the recorded history, man could lay his hands on the plants for the genetic manipulations only after the discovery of the sexes in 1694 by Camerarius who found out that like animals, plants too have the male and female organs and their reproduction follows in a well planned systematized order. For the first time in 1719, Fairchild attempted the artificial plant hybridization. He produced a number of hybrids which combined the characters of both the male and female parent. Kolreuter in 1761-1766, for the first time, reported the existence of hybrid vigour in the hybrids of Nicotiana, Dianthus, Mirabilis, Datura and other genera. He also described the contrivances of the floral structures which led to cross pollination and thus resulted in cross breeding. Later, similar observations on the presence of hybrid vigour were recorded and reported by Knight (1799), Sagaret (1826), Wiesmann (1828) Herbert (1837), and Naudin (1865). Even Mendel (1865) noticed the presence of hybrid vigour in some of the pea hybrids but he attributed it to the phenomenon of luxuriance. In an 1849 summary of Gartner's quarter century of plant hybridization, he was credited with 10,000 crosses in 700 species and 80 genera from which he obtained 250 hybrids. Many of the $F_1$ obtained in these crosses were vigorous (Smith, 1966). Knight in 1823 for the first time established that male and female parents make equal contribution to $F_1$ offspring and that segregation occurs in $F_2$ generation. Darwin in 1876 in his book "*The effect of cross and self fertilzation in the Vegetable Kingdom*" reported that the individuals obtained by out-crossing were generally more vigorous and good for better survival and has indicated that the cross pollination is generally beneficial and the self pollination is usually injurious in such crops. Much before the detailed theories of outbreeding were discovered, it was observed that the plucking of date palm inflorescences from one plant and dusting its pollen on the other was beneficial for the productivity of the palms and, therefore, wherever palms were cultivated, it was done almost ceremonially.

The most exciting advance in the heterosis breeding, however, came in with the classical work of Shull and East (1905-12), with their experiments on inbreeding and outbreeding in maize.

### (b) Contributions of Shull and East (1905-12)

Shull initially started his genetic studies on Oenothera (evening primroses) and later tried his views on maize and sunflower as well to draw broad and valid conclusions. In maize, he started his original experiment from the individual grains obtained from the four individual plants of the variety Leaming Dent at Carneigie Institute. He selfed a number of open pollinated ears and

carefully maintained their pedigree and grew them at more than one location. East made similar investigation in maize. The major findings of their work on inbreeding and outbreeding which formed the base for the heterosis breeding later, emerged to be as follows :

(i) As a consequence of inbreeding, there is reduction in the size and vigour of the lines. Each successive generation of close inbreeding still brings in the further reduction in the vigour as compared to the parental lines.

(ii) As a consequence of crossing between the weak but uniform lines, there is a restoration of the vigour which was lost during the successive generations of inbreeding accumulated over a period of several years. (Table 2.1)

TABLE 2.1

**The range of yield (bu/acre) of the various type of pollinations (After Shull 1952)**

| *Types of pollinations* | *No. of families* | *Yield (bu/acre)* | |
|---|---|---|---|
| | | *Range* | *Average* |
| 1. Selfings | 12 | 18.8 - 41.2 | 32.8 |
| 2. Use of mixed pollen grains | 12 | 58.1 - 83.3 | 73.3 |
| 3. $F_1$ crosses | 14 | 60.3 - 87.3 | 78 6 |

It was noticed that the restoration of vigour in $F_1$ or upon crossing often exceeds the total deterioration accumulated over a number of years. Shull called it to be occurring because of the stimulus of heterozygosis.

(iii) The yield data obtained from the reciprocal crosses were observed to be more or less equal and indicated that this increase in the performance of the $F_1$ crosses comes as a consequence of genes and not that of the cytoplasm.

(iv) This increase in $F_1$ performance was observed to be stable, wide occuring and could be satisfactorily reproduced over years and locations.

(v) Production of the $F_2$, $F_3$, etc. from $F_1$ indicated that there is decline in yield from $F_1$ to $F_2$ and the later generations. This reduction from $F_1$ to $F_2$ and $F_3$ in maize were observed to be 79.4 to 50.2 to 24.2 percent respectively (Shull, 1952).

Shull noticed and got the convincing proofs of the similar phenomenon in Oenothera and Sunflower as well. The heterotic effect in sunflower with respect to height was observed to be much pronounced as by crossing the prairie sunflower stocks with Russian variety which were six feet each in height, the $F_1$ was as high as 14 feet. At Gottingen in Germany, while

discussing the results of his maize investigations conducted upto 1912, in one of his lectures he proposed the term "heterosis" to replace the phrase "Stimulus of heterozygosis".

**(c) Later development in heterosis breeding methodology**

Though the presence of hybrid vigour in maize was reported in 1910, but it was not commercially utilized till 1918-19, until the double cross plan was suggested by Jones (1918). Jones (1918) demonstrated that the hybrid seed could be obtained cheaply and commercially from the double cross seeds. In terms of performance, the double crosses yield as much as the single crosses themselves. Therefore, apparently there would not be any yield loss by growing the double crosses for commercial cultivation.

The first commercial maize hybrid produced was Burr-Leaming Dent hybrid. It was produced by Jones in 1917 and was first distributed for commercial cultivation in 1922. The major contribution to the heterosis breeding methodology during 1920-1940 resulted from the discovery of male sterility in maize (Rhoades, 1933) and its commercial exploitation in the hybrid seed production of Onion (Jones and Clark, 1943), discovery and the demonstration of the use of top cross (Davis, 1927) for large scale screening and testing of inbred lines for general combining ability, and the prediction of performance of double cross hybrids from single crosses data (Jenkins, 1934). For the genetic improvement of cross pollinated populations, polycross technique (Tysdal, Kisselback and Westover, 1942), gametic selection (Stadler, 1944) and recurrent selection for specific combining ability (Hull, 1945) were developed. In 1949, Chase outlined the use of monoploids to obtain completely homozygous inbred lines in maize. He deviced a good screening technique for quick identification of monoploids through the use of marker genes.

In India with the inception of All India Coordinated Projects, heterosis breeding, especially with reference to maize, bajra and sorghum made a considerable impact on yields in these crop areas. The first of its kind was initiated in maize in 1957. Excellent germplasm resources geared in the multi-locational breeding programme and an intensive research effort was perhaps responsible for the release of 4 maize double hybrids (Ganga 1, Ganga 101, Ranjit, Deccan) in 1961 just in a span of 4 years times after the initiation of the project. Later addition in this group has been the Ganga Safed Hybrid Makka No. 2, Ganga 3, Ganga 5, Ganga 7, Hi-starch, etc. With the availability of exotic sources of male sterile lines, five promising single cross hybrids have also been released in Bajra (HB-1, HB-2, HB-3, HB-4 and HB-5) and three in sorghum (CSH-1, CSH-2 and CSH-4).

The work of Dhawan and his associates at the I.A.R.I. (1960-68) has clearly demonstrated that for a country like India, a partial utilization of heterosis in terms of breeding for composite varieties has as good possibilities as that of the double cross hybrid maize breeding programme.

Recently, heterosis breeding is being extended to newer and non-conventional areas of research. One such breeding is extension of heterosis breeding to predominantly self pollinated crops. Four good examples are the breeding hybrid sorghum, wheat, linseed and barley, etc. through the utilization of male sterile systems available in these crops. The frontiers of heterosis breeding seems to be fast changing from cross pollinated crops to self pollinated crops. The breeder is basically interested in one thing that is the improved yield whether it comes through the interactions or recombination of genes or gene systems. With the use of genetic male sterility response to DDT, cytoplasmic male sterility with restorer genes, Suneson (1963) has outlined the technique for the production of commercial barley hybrid seed. Similar reports are also now available in wheat and cotton.

The volume of noteworthy information on the understanding of the phenomenon of heterosis and its usefulness in breeding is overwhelming and is being accumulated at a very fast rate. It is obviously difficult to pool all the informations here. However, from time to time national and international symposia on plant breeding in general and heterosis in particular have done a good job in accumulating most of the advanced and valuable knowledge and ideas together. The symposia on "Heterosis" in 1950 at Ames Iowa, embodies most of the significant references on the basic understanding of the phenomenon of heterosis till 1950. This has also emphasised the importance of heterosis to plant and animal breeding. In the preface of the book Gowen wrote "Iowa has a direct, vested interest in heterosis". In the present book most of the relevant references have been reviewed and incorporated at their respective places in the ensuing chapters.

SECTION TWO

# UNDERSTANDING HETEROSIS

The extent of inbreeding in a population is often a variable parameter and is quantitatively measured by the Wright's coefficient of inbreeding denoted by (F). It is usually the probability that two allelic genes in an individual are both descended from the same gene which was present in a common

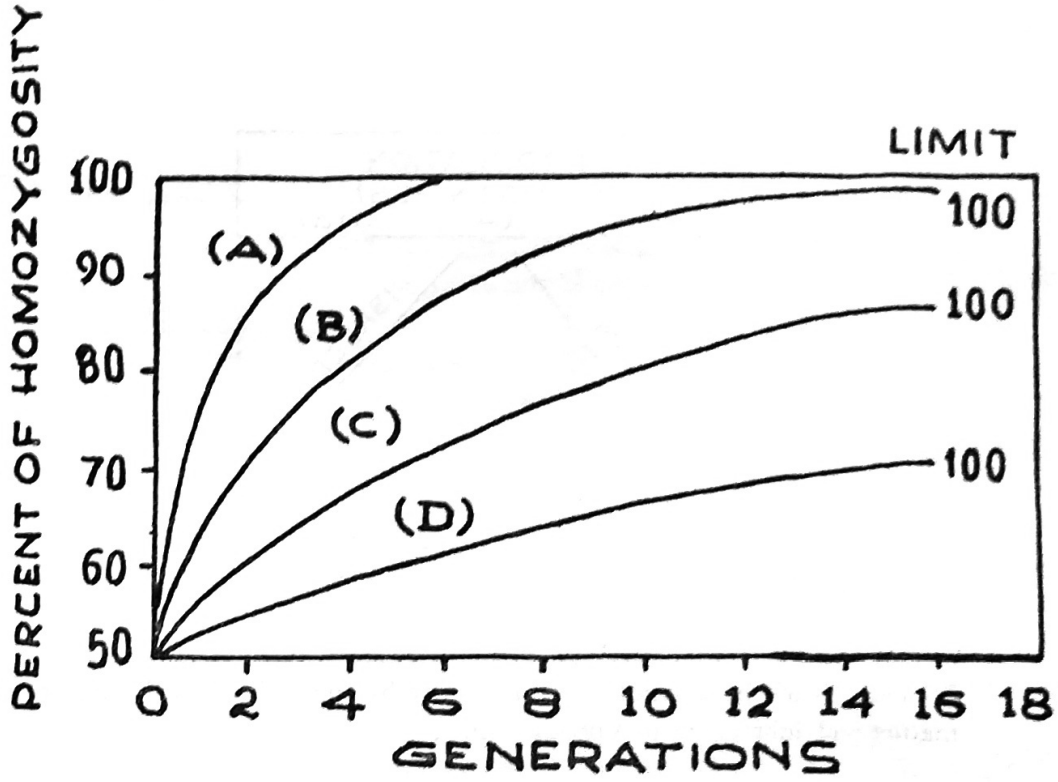


*Fig. 3.1* Percentage of homozygosity in successive generations under various systems of inbreeding (A) selfing, (B) sib-mating, (C) Double first cousins and (D) Quadrupule first cousins. Adapted from Wright (1921) and Allard (1960)

ancestor. If the frequency of two alleles A and a are in the proportion of p and q, the probability that the two uniting gametes will contain identical gene from the same ancestor in a new individual is by definition F. It then appears that an individual has probability F of being homozygous for such a gene as A or a. But since p and q represent the relative frequencies of the two alleles A and a, an individual has a probability of pF of being homozygous AA, and qF of being aa. It has also the probability of (1-F) that these two alleles did not come from the same gene in a common ancestor. In this case, they have a chance of $p^2$ to be AA and $q^2$ of being aa as in any randomly mating population. Putting together, we have the following frequencies of the three genotypes in two systems of mating :

| *Genotype* | *Inbreeding* | *Random mating* |
|---|---|---|
| AA | $p^2\ (1-F)+pF$ | $P^2$ |
| Aa | $2pq\ (1-F)$ | $2\ pq$ |
| aa | $q^2\ (1-F)+qF$ | $q^2$ |

It could be observed from the above genotypic frequencies that when the value of F is equal to 0 (*i.e.* under complete random mating) the values reduce to usual Hardy-Weinberg formula (*i.e.* $AAp^2+Aa\ 2\ pq+aa\ q^2=1$) and when F=1 (*i.e.* complete inbreeding) *i.e.* when the population is completely homozygous, the frequencies being p and q. This relationship between two systems of breeding in a population could be shown as in Figure 3.2.

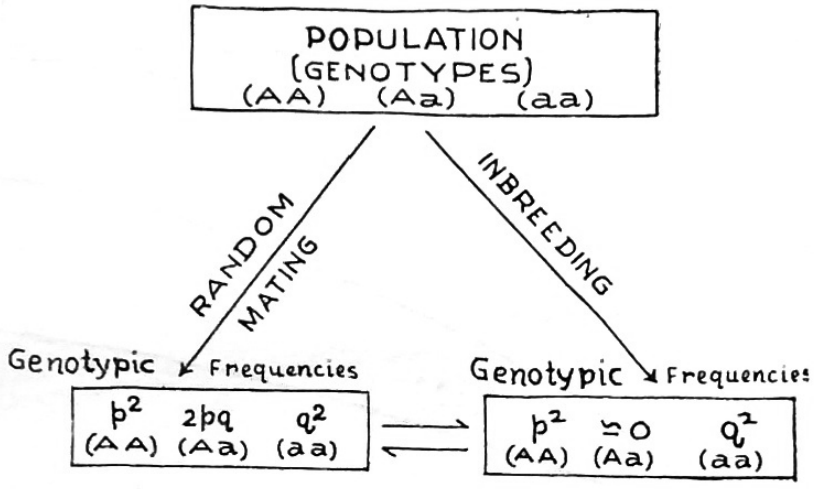


*Fig 3.2* Frequencies of three genotypes represented by one pair of alleles under random mating and inbreeding in a population.

**Consequences of inbreeding in cross pollinated crops**

Inbreeding is the common phenomenon of mating in self pollinated crops. This group of crops are biologically accustomed to inbreeding process. In such crops, high productivity is usually obtained through the production of balanced homozygous populations by manupulating favourable genes in a cultivar The cross pollinated crops are random mating panmictic populations and their reproductive biology is used to it. Any accidental, unnatural and deliberate attempt of inbreeding in such crops (Plate 3.1) is an interference and hindrance to their normal reproductive biology. It exposes heterozygosity conceal in the heterozygotes and consequently brings about a reduction or the depression in its performance. Phenotypic consequences of inbreeding, in cross fertilizing species, usually, are the reduction in plant height, size, loss of vigour, seed producing capability, fitness, yield potential and the appearance of a number of recessive abnormalities. This ultimately results in the isolation of pure breeding inbreds or lines (as usually seen in bajra). Genetically, inbreeding as stated above, brings in the rapid fixation of genotypes breaks the entire population into small non-interbreeding groups and leads to the production of inbreds. (Plate 3.2). Statistically speaking, the continuous inbreeding without directional selection, increases the total genetic variance between the families and decreases the genetic variance within the families.

The extent of inbreeding depression though observed in almost all the cross pollinated crops is, however, not similar in magnitude in all. It is considerably severe and harmful in crops like alfalfa, brown sarson, maize and some of the cross pollinated grasses. It has little or practically no ill effect in cross pollinated vegetables like cucurbits or in hemp, etc. The depression in onion, sunflower, rye, cotton, bajra, etc. falls somewhere in between these two. The high degree of inbreeding depression in crops like alfalfa and brown sarson could probably be attributed to the presence of a highly delicate balance of heterozygosity and a poor homozygous balance of genes in such crops. Such a system of delicate heterozygosity conceals a large number of recessive, lethal and deleterious genes which when exposed even with slight amount of inbreeding often brings in a devastating depression in such crops. The cross pollinated crops like cucurbits are usually grown in small pockets or in small populations as compared to the other cross pollinated crops by the farmers mostly for their local or commercial needs and evolutionally develop, rather, a better homozygous balance. Under natural conditions, they usually release some of the recessive genes and their reproductive structure almost becomes characteristics of the self-pollinated crops. The natural consequences are, therefore, comparatively less inbreeding depression owing to the less exposure of recessive genes. The moderate inbreeding depression in crops like onion, sunflower, etc. is because of the moderate fixation of these two types of homozygous and heterozygous balance in these crops.

**Genetic basis of harmful effects of inbreeding**

Mutation has been recognized as one of the major source of creating genetic variability in cross pollinated crops as well as the other plants. New mutations constantly arise. It has been noted by various workers that most of the new mutations are recessive, deleterious, detrimental, unfavourable, harmful and affect the average vigour and vitality of the populations. The increase in such mutations increases the genetic load of the population. Harmful domi nant genes are quickly eliminated as their harmful effects are immediately exposed to the natural elimination. But the harmful recessive genes usually go on accumulating in an inbred line and ultimately make it very weak. It is generally not possible to rid an inbred line of all the deterimental recessive genes with any amount of selection pressure Therefore, a depression in vigour, survival value and ultimately its yield is almost inevitable consequence of inbreeding. The inbred lines of maize particularly are the good example. They are more uniform but are very weak and poor performing Their poor performance could be safely attributed to the accumulation of unfavourable recessive genes as the consequence of intense inbreeding in them.

**Effect of self and cross fertilization on seed development**

Effects of inbreeding and outbreeding in alfalfa have been reported by Brink (1952) Alfalfa is genetically a self-incompatible material and is highly cross pollinated. Very little, if any, self pollination occurs under the natural

conditions. In the controlled experiments only fifteen per cent of ovules were fertile after selfing in contrast to 66% in cross fertilization. The frequency of ovules collapsing as a consequence of selfing were 34.4% while that in cross fertilization were only 7.1%. (Table 3.2)

TABLE 3.2

**Frequency of fertile ovules collapsing in alfalfa following self and cross fertilization (After Cooper and Brink, 1941)**

| *Type of fertilization* | *Number of fertile ovules* | | *Percentage collapsing* |
|---|---|---|---|
| | *Total* | *Collapsing* | |
| 1. Self fertilization | 314 | 108 | 34.4 |
| 2. Cross fertilization | 1211 | 86 | 7.1 |

This was attributed due to the collapse of ovules as a consequence of inbreeding. They observed that the endosperm was the seat of the inbreeding depression rather than the embryo. Embryo develops at much slower rate than the endosperm while the endosperm develops rather very aggressively. Because of inbreeding, the developmental upset takes place in the embryo primarily as a consequence of reduction in the pace of cell division. This ultimately impairs and arrests the seed development. (Fig. 3.3) The cross fertilization not only initiates the growth of embryo and endosperm but the maternal tissues like integuments also compete for growth and for getting the food. If the rate of growth of the endosperm is weak, disproportionate and larger amounts of nutrients are diverted to the integuments. This makes it almost outgrown. It becomes multilayered instead of two cell layered and becomes somewhat callus like. This further suppresses the developement of the endosperm and the seed ultimately fails to compete and dies out.

**Inbreeding Depression and breakdown of Heterosis**

One of the characterstics of the heterosis is that the increased vigour is confined only to $F_1$ generation. From $F_1$ to $F_2$ and in later generations, there is a considerable depression as a consequence of inbreeding. The extent of this depression in the same crop varies from character to character, generation to generation and also upon the types of hybrids used. East noticed that the gene fixation for height occured after 5 generations of inbreeding while yield continued to decline for 20 generations. Generally depression is rapid in first few generations and is slowed down in later generations.

Inbreeding depression was noticed as early as 1876 by Darwin. Since then a number of workers have noticed it in an array of crosses exhibiting heterosis. A summarised account of the same has been presented in Table 3.3.

The major cause of the rapid inbreeding depression from $F_1$ to $F_2$ is breakdown of the specific gene combination responsible for heterosis in $F_1$ as a consequence of segregation in $F_2$ generation. A general observation is that

single cross hybrids usually show greater yield depression from $F_1$ to $F_2$ as compared to three way crosses, double cross hybrids and most of the varietal crosses. In most of the synthetic and composited populations, extent of inbreeding depression is comparatively very low. This probably indicates the fact that yield depression is maximum in the material with low genetic bases and less in material with broad genetic constitution. These observations, however, show the importance of growing the $F_1$ hybrids for obtaining the highest heterotic yield from year to year. The $F_2$ and later generations seed can be utilized effectively only when there is very little inbreeding depression

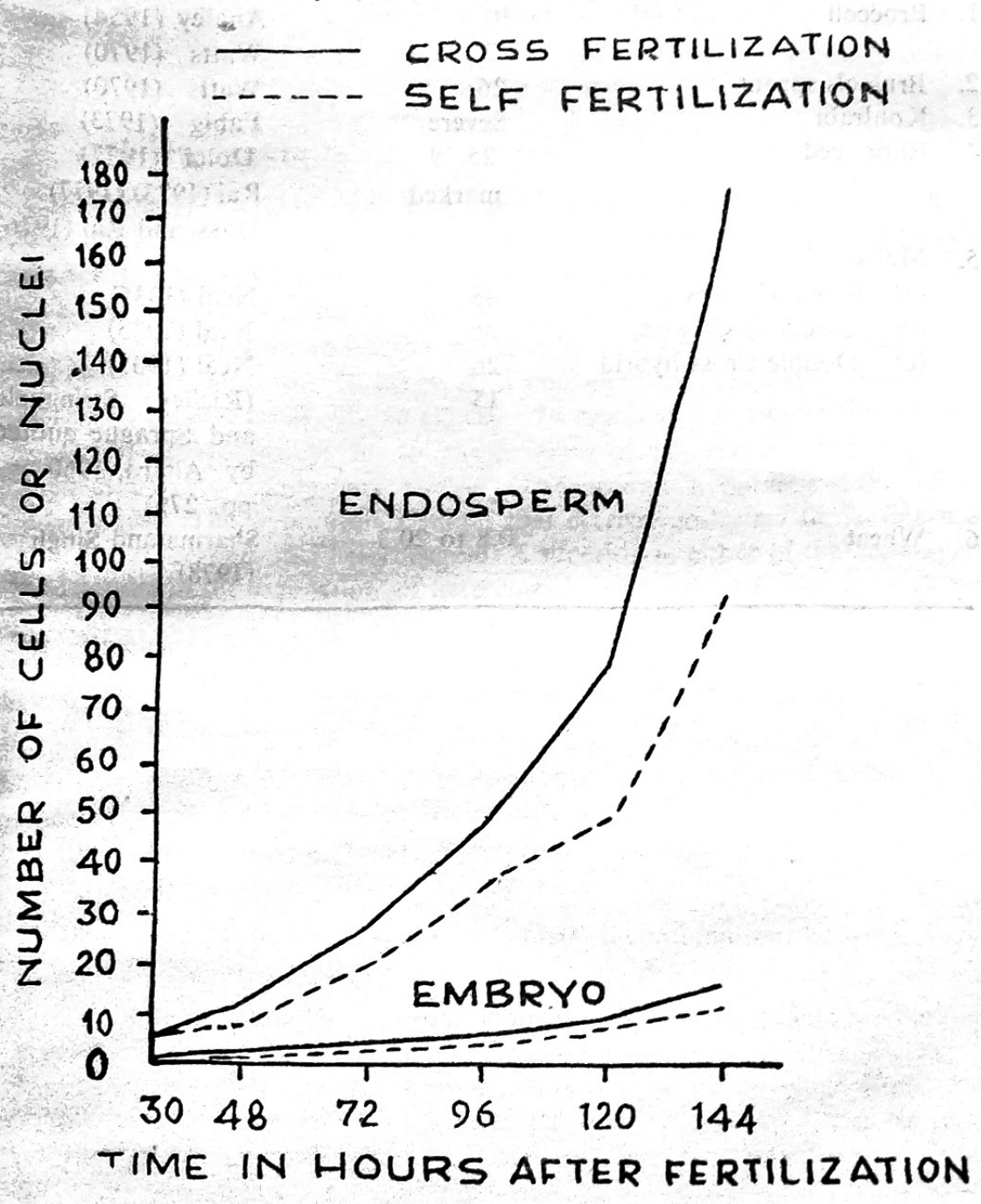


*Fig. 3.3* Cell division of embryo and endosperm under self and cross pollination in alfalfa (*Medicago sativa*). After Brink and Cooper, 1940.

just as observed in certain synthetic and composite populations and the cost of hybrid seed is economically not prohibitive.

TABLE 3.3

**Extent of inbreeding depression in yield performance of certain crop plants**

| *Crop plants* | *Extent of inbreeding depression (in percentage)* | *Authors* |
|---|---|---|
| 1. Broccoli | 10 | Anatey (1954) |
| | 3 | Watts (1970) |
| 2. Brussels sprout | 26 | Watts (1970) |
| 3. Kohlrabi | Severe | Fabig (1973) |
| 4. Rape seed | 25-29 | Doloi (1977) |
| | marked | Rai (1973) (1977) Dass and Rai (1976) |
| 5. Maize | | |
| (a) Single Crosses | 48 | Neal (1935) |
| (b) Threeway Crosses | 36 | Neal (1935) |
| (c) Double Cross hybrid | 26 | Neal (1935) |
| | 15 | (Richey, Stringfield and Sprague quoted by Allard, 1960. pp. 276) |
| 6. Wheat | 0.8 to 20.3 | Sharma and Singh (1978) |

4

# Genetic, Physiological, Biochemical and Cytoplasmic Basis of Heterosis

## 1. GENETIC BASIS

Considerable literature has now accumulated on the understanding and the analysis of the phenomenon of heterosis. Genetic machanisms responsible for the expression of heterosis have been primarily viewed from the dominance and heterozygosity of the loci concerned. There are presently two major genetic hypotheses for the explanation of heterosis. The first one is *dominance* hypothesis. It takes into consideration the association between recessiveness and unfavourable effects. In essence, it attributes the increased vigour of heterozygosity due to the covering of deleterious recessive genes by their dominant counterpart alleles. The second hypothesis is that of *over-dominance*. This hypothesis assumes that heterozygosity *per se* is important, that is, at certain loci heterozygote is superior to either of the homozygotes and results in the expression of heterosis.

### (i) Dominance hypothesis

The various basic assumptions involved in the understanding of dominance hypothesis are as follows :

(i) Genes governing vigour are dominant, beneficial in nature and the recessives are usually deleterious.

(ii) No epistasis *i.e.*, there is complete additivity of gene effects between loci.

(iii) No linkage *i.e.*, there is complete recombination between various alleles at various loci.

In Mendelian terms, the dominance could be defined as the biological phenomenon in which the dominant gene has an over-riding effect on its allele in such a way that the homozygous dominant is phenotypically indistinguishable from the heterozygote. Phenotypically it is AA=Aa. The concept of dominance as a causal force for heterosis was first proposed in 1908 by Davenport and was later supported from the researches of Bruce (1910), Keeble and Pellow (1910) and has been discussed by Whaley (1944), Gowen (1952) and Stringfield (1964). This hypothesis as mentioned before assumes

that in an inbred line, its poor performance as compared to the parental source basically stems from the handicapped development of the plant because of the accumulation of a number of unfavourable deleterious recessive genes. The inbred lines also simultaneously contain a number of dominant favourable genes which perhaps account for the variation in their performance and the variable extent of inbreeding depression. If these dominant genes are more in an inbred line the depression is usually less. When such of the two homozygous unrelated inbred lines are crossed, it is possible that at particular locus the inbred lines will differ depending on whether dominant or recessive alleles have become homozygous through inbreeding. For example, in cross of two inbred lines A and B having the genetic constitution as AA bb CC dd and aa BB cc DD respectively, the cross would be—

| A | | B |
|---|---|---|
| AA bb CC dd | × | aa BB cc DD |

$F_1$ Aa Bb Cc Dd

As is apparent, the hybrid contains more number (4) of favourable dominant genes and would be according to dominance hypothesis, more vigorous than either of the parent which have comparatively less number of dominant genes (2).

The major evidences in the support of this hypothesis have been obtained from the evidences of mutational heterosis in cross pollinated crops and the accumulation of favourable dominant genes in other plants. Keeble and Pellow (1910) with their work on peas supported this hypothesis. They crossed two plants with each one having one independent dominant gene (one governing long internode and other larger number of internodes, produced a hybrid much longer height than the either parent). The evidences obtained from convergent improvement in maize (Richey and Sprague, 1931) also supported this hypothesis.

**Objectons to dominance hypothesis**

Two major objections to this hypothesis have been :

(i) that if this hypothesis holds good, it should be possible to obtain homozygous individuals (say AA BB CC DD) which should have the same vigour as $F_1$ (Aa Bb Cc Dd).

(ii) and that in the $F_2$ population of a cross between two inbred lines (A) and (B) there should be an apparent skewed frequency distribution of the heterotic character because in such a trait the dominant and recessive loci would be distributed according to the expansion of bionomial $(3/4+1/4)^n$ where n is the number of genes involved in the expression of the character. But some of the actual evidences obtained were rather contrary to these expectations.

However, the theoretical expectations obtained by Jones (1917) and Collins (1921) have helped to remove these objections and have brought

reconciliations with these two objections. Jones (1917) indicated that with the presence of linkage, the expectation of dominance hypothesis are same as those of the superior heterozygotes because if a deleterious recessive was linked with a fovourable dominant, the heterozygote chromosomes move with homozygotes and this linked combination would not break up easily. For the disappearance of skewed distribution in $F_2$, as an objection to this hypothesis, Collins (1921) pointed out that with a large number of genes even in the absence of linkage, skewedness disappears and would be rather difficult to be detected. Further the chances of getting all the beneficial dominant genes in one type would be extremely small rather impossible if the number of genes involved in the expression of the character are very large. For example, if a homozygous plant with 30 loci are to be found in maize, detection of such a genotype in a freely segregating population would take 2,000 times the total land area of the world to grow this population. Therefore, the above two objections would hold true if only very few number of genes (1—4) are involved. But since this is usually not the case, in the genetic control of quantitative characters, the dominant genes hypothesis could be reasonably assumed to be responsible for the expression of heterosis.

**(ii) Over-dominance hypothesis**

The theory of over-dominance for the genetic explaination of heterosis have been advocated by Shull (1908), East (1908 & 1936), Stadler (1939) Gustafson (1938) and Hull (1945). Over-dominance usually has been defined as Aa>AA or aa, and has been referred to as stimulus of heterozygosis, super-dominance and heterotic interactions of alleles etc. As indicated before, this hypothesis assumes that the presence of heterozygosity *per se* is important in the expression of heterosis. The instances in which the heterozygous condition has been observed superior to either of the homozygous recessive or homozygous dominant were reported by Karper (1934), Quinby and Karper (1946) in sorghum, Robertson (1932) and Gustafson (1938 & 1946), in barley with respect to single locus heterosis. Stadler (1939) working with maize reported that heterozygous R alleles have better and darker pigmentation than either of the homozygotes and postulated that the cumulative effect of genes acting in similar way perhaps, result in the over-dominance. Evidences of single locus superiority of heterozygotes have also come in from the scute series of bristtle characters in drosophila and also from the colour pattern in the insect beetles (Ian 1946).

One of the convincing support to the overdominance theory came in from the work of Hull (1945). He worked with maize and observed that a number of inbred crosses have exceeded the sum totality of the two inbreds *i.e.*, if A line is producing 10 q/ha and B lines 12 q/ha the $F_1$ sometimes gives a value more than 22 *i.e.*, say 25, 27 etc. This could not possibly be explained with the dominant genes acting even in a completely additive manner and could perhaps be better explained on the basis of overdominance between the gene systems governing heterosis or dominance plus some gene interaction. Ofcourse this does not exclude the possibility of epistasis being present. But the

actual presence of epistasis at least in maize yields has not yet been fully established. The data obtained by Robinson, Comstock and Harvey (1949) and Crow (1952) have suggested and given the evidences of the presence of over dominance from grain yield in maize.

It could be noted here that there is considerable variability existing in the open pollinated varieties of maize. Most of the inbred lines used in the hybrids are obtained from these populations. It is now well known that the amount of ordinary selection *i.e.*, mass or ear to row selection for the accumulation of favourable dominant genes has generally not been successful in out-yielding the chosen combinations of inbred lines, extracted from the same population or varieties. Though such observations are possible on the basis of dominance with more gene interaction or epistasis but could be more clearly explained on the basis of specific combining ability of the lines or the over-dominance than merely on the basis of dominance hypothesis (Crow, 1952).

**Objection to overdominance theory**

The major objection to overdominance theory stems from the fact that most of the support and evidences obtained for this hypothesis have come in from the cases of single locus heterosis. Doubts often expressed as to what is true for a qualitative gene may not hold good for the expression of heterosis in the quantitative characters like, yield etc., or in other words in the expression of agronomic heterosis.

**(c) Similarities and differences between the two hypothesis**

The dominance and overdominance hypothesis for the explanation of heterosis both are based on the assumptions of Mendelian interpretation. Both of these agree that the out-crossing of the inbred lines leads to the recovery of vigour and its decline is correlated with the decrease in the level of heterozygosity. The major point of difference lies in their basic assumption whereby in one, it is assumed that heterosis results from the sheltering of the deleterious effects of the recessive gene by dominant counterparts, while in other it is not the sheltering effect as such but the amount of cumulative support and complimentation between the alleles which is responsible for the expression of heterosis. So far, in literature, exclusive conclusions favouring one or the other theory have not come forth. It is quite probable and possible that in an organism, both of these systems might be operating singly or simultaneously to build up the heterotic effects. As it is visualised, this phenomenon is complex and needs more understanding of gene action, of dominance, and of other fundamental genetic phenomenon particularly as they relate to the quantitative characters. As referred by Allard (1960) there is no single or simple explanation for heterosis.

## PHYSIOLOGICAL BASIS OF HETEROSIS

A chain of physiological reactions finally build-up the ultimate total harvestible products in crop plants. It is often viewed that the genes provide

only the basic blueprints and determine the operative framework for the vital plant processes and systematically govern the physiological reactions. Heterotic hybrids usually have seen to have better initial embryo weight, seedling vigour and have greater capacity to synthesize growth promoting substances, utilize and assimilate more nutrients. These physiological aspects of the plants provide better physiological efficiency to the hybrids. The non-heterotic hybrids usually have an unbalanced genotypic constitution. They have rather a number of handicapped, bottleneck genes which are limiting factors to their physiological processes and result in their poor performance. Usually when two poorly performing but genetically diverse, inbreds are crossed, they compliment and replance the physiological bettleneck genes of one another in the $F_1$ at many loci and give relatively better performance.

Contribution of heterozygosis to better physiological activity has usually been reported at three stages of plant development *viz.*, the embryo development, early seedling growth and in the later growth of the hybrid Whaley (1952). A brief discussion of these stages and their relation with heterosis has been given as follows :

**(a) Embryo development and heterosis**

Ashby (1930, 1932 and 1937) attributed that the heterosis is associated with a greater embryo weight and size. In some instances, the initial high embryo weight has been observed in a relatively large seed, but Whaley (1950) observed that this is not universal and usually there is no positive correlation with size and vigour of the seed. He observed that some of the double crosses have large seed, vigorous $F_1$ but had only medium sized embryos. Copeland (1940) also contradicted this view and pointed out that the size of the embryo is determined not only by the genotypic back-ground of the hybrid but also by the nutritional supply of the female parent as well and concluded that the embryo growth may or may not be related to the expression of heterosis.

**(b) Early seedling growth and heterosis**

It has been usually observed that heterotic hybrids attain their physiological superiority within first two hours of germination. It has been attributed that the rapid early seedling growth is associated with heterosis (Ashby, 1930, 32 and 36; Hatcher, 1939 and 1940; and Luckwill, 1937 and 1939). A number of studies of early growth during the first two weeks of germination have shown that the heterotic seedlings show higher growth rate than the inbreds. The basic physiological activity during the early seedling growth is the formation of enzyme patterns, the translocation, transformation and utilization of stored food material in the seed and then in the building-up of the active protoplasmic base for further physiological activity. At this stage the heterotic effect is generally apparent and visualised in the acceleration of growth rate and in bringing up a differential level of metabolism which ultimately results in the quantitative differences in size, vigour etc. Chery, Hageman, Rutgar and Jones (1960) reported larger quantities of embryonic nucleotides and

greater synthesis of RNA in the hybrids as compared to the inbreds and suggested that this perhaps may be the reason for faster growth and development of the hybrids.

Sprague (1936) agreed with the above view but failed to demonstrate a higher growth rate for the hybrids from the late seedling stage to maturity of the plants.

### (c) Later growth and heterosis

A number of studies on the later growth and heterosis have tried to establish mostly the basic physiological differences between the inbreds and the hybrids. It has been visualised that the hybrids usually give better performance than the inbreds primarily because they have better and profuse root system (Kisselbach and Weightling, 1935) which helps better absorption of minerals and nutrients, better capacity to utilise major plant nutrients (Nitrogen, Phosphorus and Potash) Smith (1934), Harvey (1939) greater catylase and ∝ amylase activity in root tips (Harvey, 1939), Sarkissian, Kissinger and Harris (1964), better capacity to synthesize growth promoting substances (Robbins, 1941) and finally a much higher level of metabolic activity (Whaley, 1952; and Sarkissian *et al.*, 1964) (Fig. 4.1).

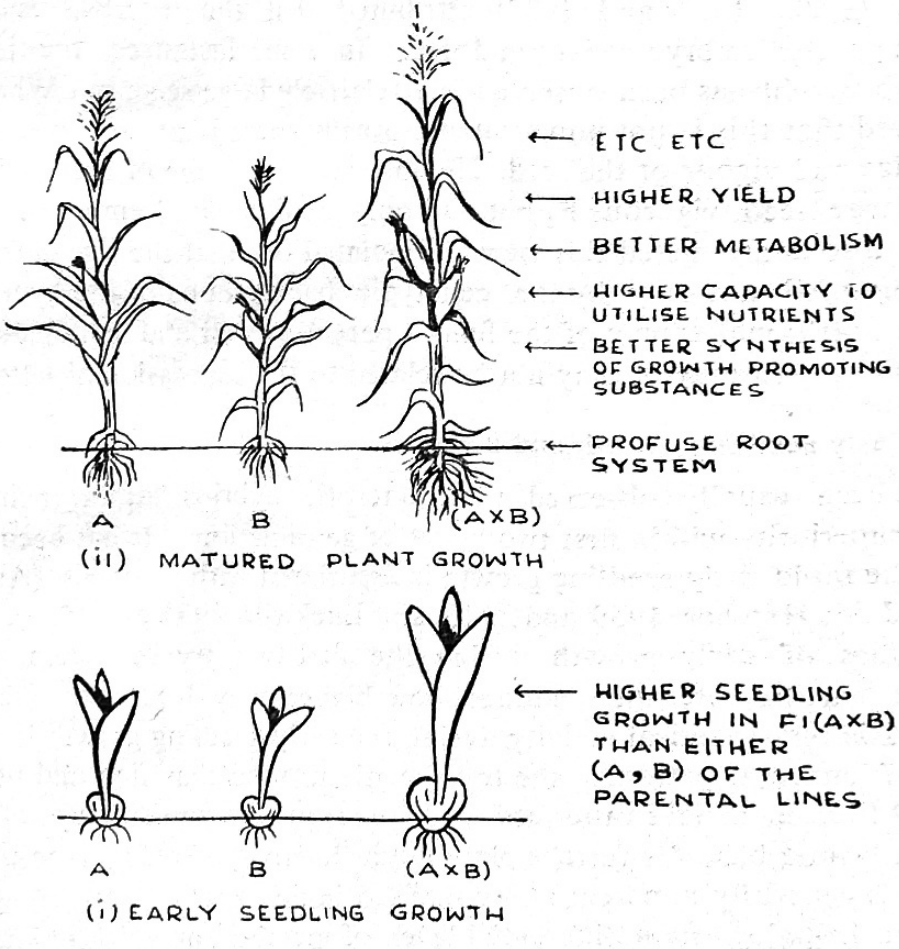


*Fig. 4.1.* A general comparison of the parental lines (A and B) and the heterotic hybrid (AxB) in maize from physiological point of view.

Further insight into the detailed physiological-biochemical understanding of heterosis has recently been obtained from the understanding of obtaining superior growth rate in the heterotic hybrids through efficient mechanism of complimentation of usable chemical energy through ATP synthesis in mitochondria, the chemical power houses of the cells. Mc-Daniel and Sarkissian (1968) and Sarkissian (1972) observed that the mitochondria extracted from the hybrids showed considerable polymorphism and exhibit distinct complimentation and heterotic effect with respect to the oxygen uptake and phosphorylation.

### (d) Mitochondrial complementation

Sarkissian and Srivastava (1967) studied mitochondrial complementation in the mitochondria extracted from the scutella of the maize seedling from the various lines, single and double crosses made between the lines Wf 9, Oh 43, 45, West Virginia 5, and 12. They reported that the mitochondria isolated from the heterotic hybrids exhibit heterosis with respect to oxygen uptake and phosphorylation while the mitochondria obtained from the non-heterotic crosses do not exhibit this phenomenon. They mixed a 1 : 1 proportion of the mitochondria obtained from the parents of the heterotic hybrids and observed that these together approached the level of mitochondrial activity of the heterotic hybrids while the mixture of the mitochondria obtained from the parents of non-heterotic hybrids did not. Complementation was considered positive if the activity of the mixture was greater than the expected average of the parental mitochondria. Negative complementation occured when the activity of the mixture of the two parental lines was less than the average of the two. It, thus, appeared apparent that the heterosis with respect to the seedling growth rate in maize is correlated with its mitochondrial activity since the mitochondrial oxidative phosphorylation is considerably important in providing usable energy for growth rate resulting from the interaction of two polymorphic mitochondria obtained from genetically two diverse parents.

The above workers while trying to explain the mechanism of complementation have shown that although it is not very clear as to how this complementation occurs, however, it appears likely that a particle to particle contact between the two diverse polymorphic mitochondria occurs as a result of production of a complementary specifically efficient intermediary in the liberation of the usable energy in the electron transport chain for the efficient energy transfer in Kreb's cycle. They concluded that an extensive study and analysis of mitochondrial complementation may, however, provide an operational mean of the study of biochemistry of heterosis and may be useful in determining the potential combining ability of parental lines in a hybrid breeding programme.

## (iii) BIOCHEMICAL BASIS OF HETEROSIS

### (a) Model of single gene heterosis

Models of single gene heterosis with respect to biochemical characters

have furnished further insight into the understanding of heterotic. These evidences primarily throw light on the production of heterotic effect as a consequence of complementation or by providing the production of a balanced metabolic interaction which provides the hybrid advantage. The effect of internal biochemical factors on the heterotic expression in root growth in tomato has been reported by Robbins (1941, and 1952). He excised root tips from the varieties, Johannesfeuer and Red Currant as well as their $F_1$ hybrid, and grew them in the solutions supplemented with growth promoting substances, pyridoxin and nicotinamide. It was observed that Johannesfeuer showed greater response to pyridoxin while Red Currant to nicotinamide suggesting thereby that these two varieties were not able to synthesize these growth factors on their own and their root growth was limited because of this bottleneck factor. However, the hybrid was able to grow even in the absence of these two growth factors and was not deficient *i.e.*, it was able to synthesize them on its own. These observations suggested that the expression of heterotic advantage is an expression of the activity of the favourable combination of biochemical growth factors produced as the consequence of complementary gene action in the hybrids.

An example of the intragenic complementation and the production of a different effect (which in essence is analogous to overdominance) have been given by Srb and Weyman (1966) with respect to the peak gene in nurospora. A number of recessive peak mutants occur in nurospora and usually give non-linear asci arrangement in the ascus. But when the different peak mutants are crossed, most of the combinations produce linear asci (Fig. 4.2).

Complementation in nurospora heterokaryons have also been given by Beadle and Coonaradt (1944). They observed that two mutants, one of which was unable to synthesize nicotinic acid and was unable to grow on media lacking this acid and the other was unable to grow on media lacking pantothenic acid because mutation at this locus blocks the synthesis of this acid. But when these two were put in a heterokaryon and were grown on the same minimal medium without the addition of these two growth factors, luxuriant mycelial growth occurred. This biochemically corresponds to the example given by Srb and Weyman (1966) with respect to peak mutant in nurospora.

### (b) Allelic diversity and the production of balanced metabolic interactions

Heterosis due to heterozygosity at one locus as a result of balanced metabolic interaction has been reported by Emerson (1948) with respect to the suppression of the sulfonamide requiring character in nurospora. He obtained a mutant which required additional supply of P-aminobenzoic acid (pab) to grow on the medium. He obtained another mutant which required the additional supply of sulfonamide (Sfo) in the growth medium to grow. As is now well known, sulfonamide usually forms a complex substance which counteracts and regulates the excessive production of p-aminobenzoic acid which if unchecked or uncontrolled in biochemical path-way is poisonous to nurospora. He crossed these two and obtained a double mutant carrying both the

p-aminobenzoic acid (pab) which blocks the synthesis of pab and sfo which needs extra supply of sulfonamide. When he made a heterokaryon of this double mutant pab/sfo with a mutant which carries sfo and the original type of pab which produces and does not require pab, *i.e.*, (pab sfo pab+sfo) this

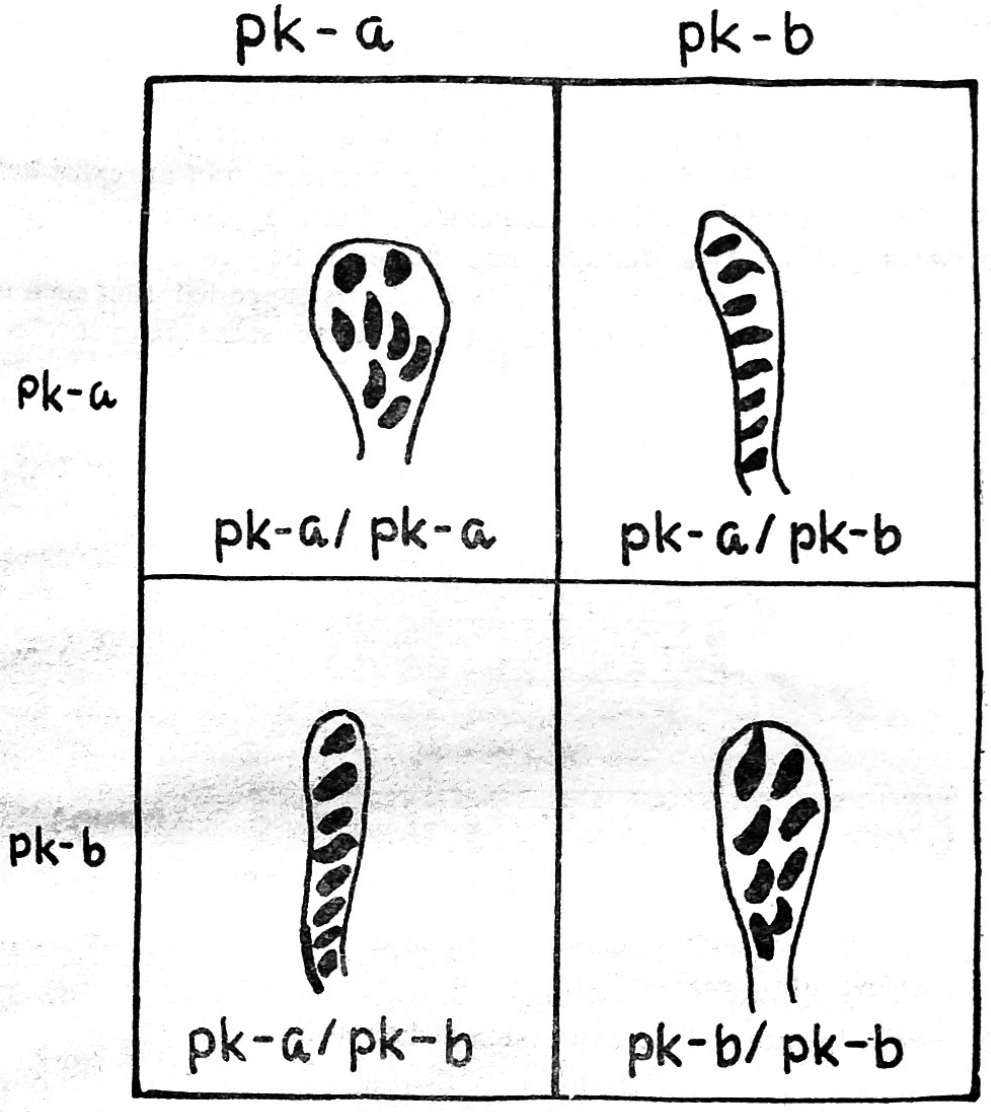


*Fig. 4.2.* Intragenic complementation at the peak locus in *Nurospora* (After Srb and Weyman 1966).

heterokaryon grows on minimal medium having neither sulfonamide nor para-aminobenzoic acid. Emerson interpreted this instance of one gene heterosis as a consequence of "growth resulting from a balanced obtained by the production of para-aminobenzoic acid by one type of nucleus and the lack of production or counteraction by other to give a tolerable optimum amount of pab thereby giving it most favourable dose of para-aminobenzoic acid and by providing a favourable metabolic balance sufficient for growth". In other words the heterozygote (pab+/pab) performed better than either of the homozygotes.

### (c) Production of hybrid substances

Irwin and Cole (1936) and Irwin (1952) observed the specificity in gene effects in heterozygotes and have reported the production of hybrid substances in pigeon-dove hybrids. A cross was made between males of an Asiatic species the Pearlneck (*Streptopelia chinensis*) and the domesticated Ringdove female (*Strepto risoria*). The corpuseles of the hybrid had almost all the substances common to each of the parental species alongwith a complex of antigens substance known as the hybrid substance (Fig. 4.3). The production of the hybrid substance is specific to this hybrid and is not found in other hybrids in crosses of pigeons and doves. It was suggested that the production of an extra hybrid substance in such a hybrid is the consequence of heterozygosis and the interaction between genes. This, though, had not ultimately reflected in terms of heterosis in quantitative characters. However, it was suggested that such comparable specificity of gene effect might well have some relevance to the phenomenon of heterosis.

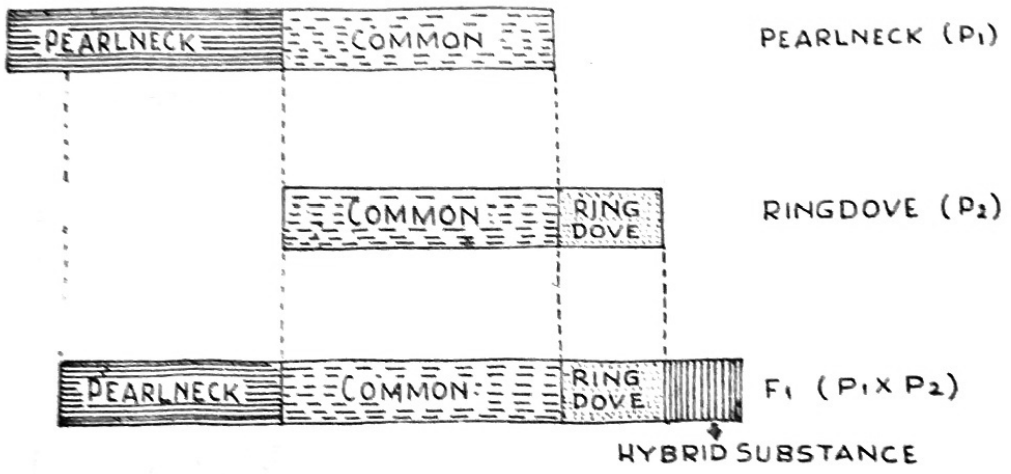


*Fig. 4.3* : Production of hybrid substance in the interspecific cross between Pearlneck x Ringdove (After Irwin 1952).

### (d) Nitrate reductase and the expression of heterosis

Since plant breeders are basically interested in grain yield in crop plants which in turn often constitutes of the bulk of grain protein, carbohydrate etc. it is rather interesting to study the synthetic processes of these biochemical products in the hybrids and the inbreds. The enzyme nitrate reductase (NR) is predominantly needed in the nitrogen metabolism and plays a major role in the conversion of nitrates into the ammonia. This in turn is utilised in the production of various amino acids which build up the total protein. Therefore, nitrate must be effectively reduced prior to its elaboration into amino acids. The reduction sequence is initiated by this enzyme. It is also a major control point for the systems that supply reduced nitrogen to the plant. Schrader and Hageman (1965) analysed the nitrate reductase activity in maize hybrids Hy 2×Oh 7 and WF 9×C 103. The former hybrid showed nitrate reductase activity much higher than the latter during reproductive phase *i.e.*, at

the time of pollination. The analysis of the plant at the end of the season showed that hybrid Hy 2×Oh 7 contained more protein and less unreduced nitrate than the parents of the hybrid WF 9×C 103. Other workers have shown that increases in nitrate activity is associated with the increased protein content and decreased nitrate content (Hageman *et al.*, 1961).

The production of NR in an inbred has been observed to be a genetically controlled character but is considerably affected by the seasonal variations. Zieserl and Hageman (1962) and Zieserl *et al.*, (1963) evaluated the NR content of 47 inbreds and classified them into high and low NR activity lines. The inbreds classified as high and low in NR were then crossed to produce high×high (5 in number), high×low (10 in number) and low×low (7 in number) hybrids (Schrader *et al*, 1966). The mean of NR in three high×high NR hybrids were equal to the mid-parental value while in the other hybrids they were significantly less than the mid-parental value. No heterotic level was observed in any of the high×low hybrids. Only in one of the 10 high×low hybrids (*i.e*, Oh 2×Oh 7), the hybrid differed significantly from the mid-parental value. But none of the cases of heterosis in NR activities was found in this group. In the case of low×low group, only 1 hybrid (*i.e.* B 14 ×Oh 43), was significantly high in NR activity than either of the parents. The other low×low hybrids, however, showed no heterosis. A general observation was that even though heterosis for NR activity was observed, the heterotic hybrids were really in the intermediate range as they do not approach the activity of certain high inbreds (Hageman *et al.*, 1967). Yet all the hybri- studied showed heterosis for grain yield regardless of the level of NR in them. This indicated that though NR may be a very important factor in nitrogen metabolism but that is not all needed for the ultimate agronomic or the yield heterosis. The overall heterosis could be obtained by the sum total of all the complex metabolic and physiological function of the enzymes present in the maize plant taken together. It is now rather well known that the end product is the net result of a complex series of chemical reactions and in the whole range any one may be a limiting factor (Hageman *et al.*, 1967).

### (e) Theoretical insight into the complexities of heterosis

A theoretical insight to illustrate the need for a balanced system and an integrated control of these systems to bring high production efficiency (with respect to the production of a complicated automobile car, and its relevance to the integration of enzymatic systems that might be operating with respect to the phenomenon of heterosis) has been given by Hageman *et al.*, 1967. (Figure 4.4).

As given in Figure 4.4, the higher production of cars depends not only on the more raw material or more production by one assembly line but by the properly coordinated rate of operation in all the assembly lines. In a similar way, the heterotic effect in a single enzyme system in root zone, meristem, seedling or elsewhere alone could not be the basic cause of total heterotic vigour. The heterotic effect, as a matter of fact, lies in the efficient, integration

and the timely operation of the essential individual enzymes and the optimum balance of the enzymatically catalized metabolic systems. The $F_1$ hybrids usually possess a more favourable genetic constitution and thus produce a better enzymatic balance for overall enzymatic efficiency than do

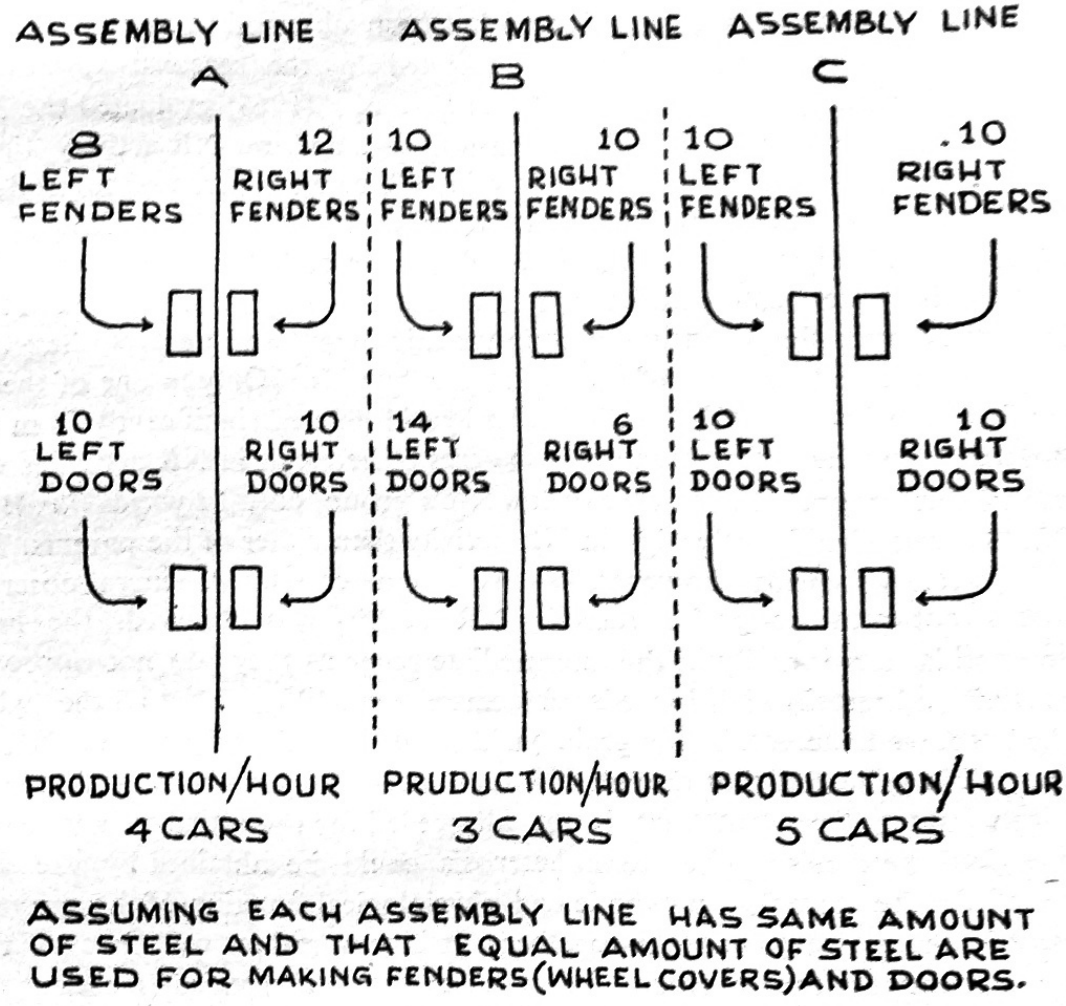


*Fig. 4.4* : Illustrating the need for balanced and integrated control system of car production to explain the phenomenon of heterosis. (After Hageman, Leng and Dudley, 1967).

either of the parents. Heterozygote has been observed to be usually intermediate in enzymatic activity which, as a matter of fact, is adequate for most of the major metabolic processes and may actually be more effective for overall metabolism. At enzymatic level, allele 'A' may specify one enzyme and allele 'a' a different enzyme. Thus the hybrid could possess both enzymes or enzyme forms which are available in one and separate inbreds. The inbred on the other hand rather has a fixed type of enzymatic balance due to inbreeding either at very high or at very low or at an otherwise ineffective level of activity. When these are there in the hybrids, the $F_1$ usually has the better metabolic efficiency and wherever this balanced situation exists, this might be resulting in the ultimate expression of the heterosis.

## (iv) CYTOPLASMIC BASIS

### Role of nucleus × cytoplasmic interaction in the expression of heterosis

Cytoplasm is a limpid fluid which is usually transmitted through the female line to each of the daughter cells taking part in the development of the organism. It is usually rich in RNA, and contains the cytoplasmic bodies like mitochondria, centrioles etc. So far we had been considering the role of genes exclusively for the expression of heterosis. However, a number of evidences are now available which indicate that heterosis may result from the interaction of genes and cytoplasm. Ruenbenbauer (1962) proposed that the yield of an $F_1$ hybrid is a function of both the genome and the cytoplasm. Jones (1952) had indicated the presence of the interaction of genes and cytoplasm in a maize cross WF 9 × California rice pop with respect to tillering habit. Inbred WF 9 produces no tiller. California rice pop inbred produces an average of 4.1 tillers. The cross CRP California rice pop × WF 9 gave one tiller while the reciprocal gave 2.2 tillers per plant. Further evidences are now available which indicate that transmissible differences with cytoplasm in the expression of genotypes are important in the expression of heterosis. Dhawan and Paliwal (1964) have reported the role of cytoplasm in the manifestation of heterosis in maize. They made crosses between Sikkim Primitive (SP) 2, a primitive Indian maize race and Col. an advanced US maize race. The data (Table 4.1) revealed that along with nucleus, cytoplasm also plays an important role in the expression of heterosis specially when the races being crossed are of very divergent origin, and may have a degree of inhibition in the heterotic expression of the characters.

TABLE 4.1

**The yield performance of reciprocal crosses between SP 2 and Col. in maize (after Dhawan and Paliwal, 1964)**

| *Pedigree* | *Yield kg/ha* | *Yield % of Col.* | *Yield % of SP 2* |
|---|---|---|---|
| 1. SP 2 | 615 | 45 | 100 |
| 2. Col. | 1,375 | 100 | 224 |
| 3. SP 2 × Col. | 639 | 46 | 104 |
| 4. Col. × SP 2 | 2,972 | 216 | 483 |

They, however, concluded that in a heterosis breeding programme, one should not only look for superior hybrid nuclei but also ascertain the utilization of superior sources of cytoplasm.

It is now clear from the foregoing discussions on the various facets of heterosis that there is yet no simple or single explanation of heterosis. It appears that heterosis results from the combined actions and interactions of a number of genes or gene systems and could be as complex as the quantitative

characters themselves. There are indications that both dominance and over-dominance situation singly or simultaneously might be operating in the expression of heterosis in the same organism. Physiological and biochemical observations with hybrids and their parents have indicated that the heterotic advantage is basically the result of the increased physiological and or metabolic activity as a result of balanced integrated processes, brought about in the heterozygote. Biochemically, it might be resulting from an optimum integrated control of enzymatic and other chemical reactions undergoing in the plant brought about by a balanced heterozygosity in the $F_1$. Though not largely evident, however, expression of heterosis sometimes might be conditioned or influenced by the cytoplasmic background of an organism as well. Finally a clearer picture of heterosis could better be obtained from the further probe and understanding of the mechanics of basic gentic phenomenon like dominance, complementation and other such fundamental physiological and biochemical phenomenon as they relate to the study of quantitative genetics and the expression of heterosis in both micro as well as the macro-organisms.

5

# Basis for Superiority of Heterozygotes

In terms of population genetics, heterosis could be defined as the genetic phenomenon arising out of the fixed amount of heterozygosity which provides the hybrid population the optimum and the best adaptive value in a given set of conditions. In inter-breeding plant populations, hybridity or heterozygosity is often required for the better adjustment of the species to varied ecological and environmental conditions and for obtaining better adaptation, survival, breeding and the fitness values.

**Fitness and heterosis**

Generally speaking, fitness is the relative ability or the efficiency of a genotype to produce viable off-springs in the next generation. In other words, if a particular genotype say A is able to produce more number of viable seeds or plants per generation, for example 1000 as compared to other genotype B of the same species which produces comparatively less numbers (say 450) of seeds per generation, then A will be called to have better fitness.

In the case of cross-pollinated random mating populations, outbreeding mechanisms like heterostyly, dichogamy, male sterility, self-incompatibility; monoecism and diocesm etc. usually aim at producing considerable hybridity as consequence of favouring crossing and inter-crossing generation after generation. During this process, it is usually observed that a heterozygote performs and survives better if it catches a balanced heterozygote condition during the random mating process either through a balanced gene combination or through the gene mutation followed by its selective advantage. The balance heterozygote obtains a hybrid optima, a fine adjustability and genotypic manoverability in a given set of conditions. It usually kicks better vigour and bio-chemical metabolic versatality. It has also better resistance to odd natural conditions, high seed producing capacity and usually a better fitness. The usual consequences are then that such a combination is better viable and produces more seeds or off-springs and thus the number of seeds or the yield of individuals increases.

In some of the insect populations, it has been frequently observed that some of the hybrids which have selective heterozygote advantage at certain locus, show high viability and better reproducing capacity. Under natural conditions, this has relevance to their survival. Such an example of balanced

polymorphism and its advantage has been given as follows :

**Balanced polymorphism and heterozygote advantage**

A case of balanced polymorphism giving rise to a heterozygote advantage has been observed and reported by Capsari (1950) and quoted by Buzzati Traverso (1952) in moth (*Ephestia kuhniella*). Capsari (1950) reported that in natural populations of this moth, two types of individuals exists, one with brown testes colour, and other with red testes colour. Brown is dominant over red. With respect to viability, the heterozygote (Bb) was observed to be superior to recessive heterozygote (bb) which in turn is better to dominant heterozygote (BB) or Bb>bb>BB. With respect to mating ability, the situation is Bb>BB>bb rather reverse. It is thus clear that the recessive for testes colour acts as dominant with respect to viability and the dominant testes colour acts as the dominant with respect to mating behaviour. Here it is apparent that though the bb and BB had varied dominance relationships with respect to viability and mating ability but the survival of the moth in the natural population occurs as a consequence of any of the two relative abilities. The heterozygote is either equal or superior to both. The net result, therefore, is its survival and selective advantage. Though he could not get a clear-cut case of heterosis with respect to other quantitative characters in the heterozygous individuals, he, however, concluded that perhaps this is how heterosis could be accounted for by the behaviour of two visible alleles in a heterozygous condition.

**Heterozygote advantage in Polygenic attributes**

Selective heterozygote advantage with respect to polygenic traits like fertility in terms of egg producing capability, rate of development, longivity and body size has been reported by Buzzati Traverso (1952) in fruit fly (*Drosophila melanogaster*). He started selection programmes with two populations of drosophila. The one was with white bar eye ($P_1$) and the other was with normal red round eye ($P_2$). These two populations $P_1$ and $P_2$ differed quantitatively with respect to the quantitative characters mentioned above. He continuously selected them for quite a number of generations (100). But by the end of 30th generation of selection in both population they were normal with respect to their eye colour and the visual mutation was lost. Natural selection in $P_2$ favoured high egg producing capacity and longivity for quite a number of generations. After 100 generations of selection the means of these quatnitative characters in the selected population were six times better than that of the mean of the original population. When the $F_1$ between these two selected populations was obtained the values obtained were equivalent to the values that would have probably been obtained after more than 100 generations of selection for these characters. He explained this situation in $F_1$ primarily because of selection during 100 generations might have picked up a genotype with new combination of polygenes and might have preserved certain amount of favourable heterozygosity or combination of polygenes which have

better adaptive value which upon crossing might have got maximised in $F_1$. This could have helped obtaining the observed optimum hybrid advantage.

**Physical basis of heterozygote advantage in crop plants**

*Component analysis of yield heterosis*

Yield in crop plants with which the plant breeders are most interested, is a complex attribute. It is basically a product of the action and interaction of a number of characters. Some of these are good seedling vigour, high tillering capacity, greater photosynthetic efficiency, higher number of fruits/pods per plant, seed per fruit and ultimately a good seed size. These attributes individually are termed as yield components. They are usually inter-related and together form the basic physical architecture of the plant yield. Usually a change or superiority in any one of them reflects and manifests in the ultimate yield heterosis in the hybrid population. Therefore, it needs to be understood for improving or maximizing yield heterosis in hybrid populations.

Leng (1963) analysed the contributions of various yield components on the expression of yield heterosis in maize. He reported that the number of kernels per rows has major effect while the kernel weight has only small effect in expressing it. The attributes number of kernel rows per ear, and the number of ears per plant were observed to have genetically no influence on the expression of yield heterosis in this crop.

Quinby (1963) reported heterosis in sorghum. He observed that the heterotic hybrids in general were characterized by the higher tillering capacity, earlier blooming period, greater height and the yield of the grain and forage as compared to the parents. The heterotic hybrids usually resulted in greater stalk diameter wider but not always the longer leaves, higher threshing percentage and more number of seeds per plant. Number of seeds per head of single stalked hybrid in the three best heterotic hybrids were 77, 68 and 24 per cent above the mean of the parents. An increase in the seed number contributed more to the increased yield of the hybrid than any other single character. Another character which was observed to contribute significantly to yield heterosis in such crosses was the number of tillers per plant. Faster cell division was believed to be the basis of the heterotic manifestation of these characters. More or less parallel observations have been observed in wheat (Sharma and Singh 1978).

Marani (1963, 64 and 67) made diallel crosses between three varieties each of *Gossypium hirsutum*, *G. barbadense* and among the species themselves. He observed that the yield heterosis in the hybrids resulted basically through an increase in the number of bolls per plant in interspecific crosses while through an increase in the number of bolls per plant as well as the boll size in the intraspecific crosses. The relative magnitude of heterosis was more in the interspecific crosses than the intraspecific crosses. He suggested that for the hybrid production on commercial scale, the *G. hirsutum* should be selected for high lint index and *G. barbadense* for high number of bolls per plant and

high lint percentage. More or less similar observations in cotton have also been reported by White and Richmond (1963) and Miller and Lee (1964).

Dass and Rai (1972) studied heterosis in 9 intervarietal crosses of toria (*Brassica campestris* var. toria) and analysed the contribution of yield components in building up of the yield heterosis in the various hybrids studied. Four hybrids namely DS 17 M×B 54, DS 17 M×$T_9$, DS 17 M × Local and B 54×$T_9$ showed significant yield heterosis. However, out of these only one (DS 17×Local) showed significantly higher number of siliqua per plant. Two hybrids (B 54×$T_9$) and (B 54×Local) showed significantly higher number of seeds per siliqua, though there was a general superiority of the $F_1$ hybrids over the parents with respect to these characters. They concluded that the yield heterosis in the hybrids studied could have resulted from an overall combination of the favourable expression of these characters together. Such a situation of the yield heterosis has been often referred as a type of "Combinational Heterosis"by Hagberg (1952) and William and Gilberts (1960) and has also been observed and reported by Ramanujam, Rohewal and Singh (1963) in gram (*Cicer arietinum*).

6

# Genetic Diversity, Combining Ability and Heterosis

## (i) Origin of genetic diversity

The major sources of the origin of genetic diversity in plants could be enumerated as mutations, recombination and polyploidization whether they are accomplished through the natural agencies or the artificially controlled conditions. A basic skeleton of the probable sources of the origin of genetic diversity has been presented in Table 6.1.

TABLE 6.1

**Major sources of the origin of genetic diversity in plants**

| *Sources* | *Types* |
|---|---|
| 1. Mutations | Spontaneous and Artificially induced |
| 2. Gene recombinations | Hybridization (both Natural and artificial) in terms of<br>a. Intervarietal<br>b. Interracial<br>c. Interspecific (rare) and<br>d. Intergeneric (very rare) |
| 3. Polyploidization | Natural and artificially induced. |

Natural or spontaneous mutations have been one of the major sources of the genetic diversity under natural conditions but usually they are observed to be rather much slower than the artificially induced ones. The latter has been observed to create tremendous genetic variability in rather a short span of time and is now becoming almost a thoroughfare for creating new genetic diversity in economically important crop plants. Macro mutations have pronounced effect and could be easily picked by visual observations while the micro or small mutations go on accumulating over large ranges of time and add to the differentiation and diversification in the various crop species.

Hybridization (natural and artificial) has been another very important and the potential source of creating genetic variability and is conventionally a major source of accomplishing gene recombinations. It has now been clearly indicated that mutation as a source of variability is rather too slow for

rapid bursts of evolution and that the variability of considerable magnitude could be achieved through hybridization (Stebbins 1959). As is now well known some of the wide crosses bring together most divergent germplasm and the genetic variability increases quite rapidly. But often much divergent crosses *i.e.*, mostly at the level of interspecific or intergeneric crosses usually result in the problems of genetic sterility, physiological imbalance and other developmental disturbances in most of the sexually producing crop species. But in the case of some asexually propogated crops, simple varietal hybridization at times does not generates required variability. In crops like potato, sugarcane, banana etc., which usually function with the unreduced gametes and can tolerate the shock of massive doses of alien germplasm, it is possible to obtain even wider crosses *i.e.*, interspecfiic or intergeneric crosses.

Polyploidizatoin of various forms euploidy (auto and allo polyploidy) and aneuploidy of various kinds has been another source of genetic diversity in a group of crop plants. Additions and losses of chromosomes or whole set of chromosomes are important sources of genetic divesity. Since euploidy and aneuploidy result in altered number of genes and sometimes in altered genetic composition, they often cause the morphological or the physiological variations in the organism. The relative rapidity with which this process could take place in nature depends upon the natural circumstances and the degree of sudden change. Many a plants change rapidly and provide polyploid types better adapted to altered natural and ecological conditions and thus survive better than others. Natural polyploidization has been responsible for evolution of di or multigenomic species in a number of food, fibre and forage crops. Some of the diversification in the highly evolved crops like, wheat, cotton, potato, mustard etc. could be easily attributed to this source of genetic diversity in plants.

### (ii) Introgressive hybridization and its role in the expression of heterosis

Introgressive hybridization is a type of hybridization in which the genes or gene blocks of one species or very distinct type are added to the genetic background of another species or distinct type through crossing and often through repeated back crossing.

### (a) How introgression of genes takes place

In natural cross pollinated fields, some occassional intercrossing between the distantly related races or species takes place. Usually 50% of the alien germplasm of a stock is often sufficient to upset the physiological or the genetic balance of the female plant which has adapted to the native environment over thousands of years of natural selection. But somehow if this $F_1$ survives and that it survives in many a cases inspite of the prevailing sterility if there is full flowering time synchronization, than this germplasm is passed on to the native plant through repeated crosses and often through the series of natural back crosses. In the final endproduct only the best adapted favourable genes or

rapid bursts of evolution and that the variability of considerable magnitude could be achieved through hybridization (Stebbins 1950). As is now well known some of the wide crosses bring together most divergent germplasm and the genetic variability increases quite rapidly. But often much divergent crosses *i.e.*, mostly at the level of interspecific or intergeneric crosses usually result in the problems of genetic sterility, physiological imbalance and other developmental disturbances in most of the sexually producing crop species. But in the case of some asexually propogated crops, simple varietal hybridization at times does not generates required variability. In crops like potato, sugarcane, banana etc., which usually function with the unreduced gametes and can tolerate the shock of massive doses of alien germplasm, it is possible to obtain even wider crosses *i.e.*, interspecific or intergeneric crosses.

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gene blocks directly or indirectly supporting the adaptation or the yield performance or better resistance toward natural conditions express themselves in the native germplasm. Thus a few new genes otherwise alien but favourable, are introduced in the population. This quite a times makes the population better suited for adaptability and survival under natural conditions. Mangelsdorf (1943) made a survey of such phenomena existing in maize fields in Mexico where teosinte usually grows as a weed. Out of 500 plants, he randomly scored and examined, 288 were just like maize, 219 were like teosinte and 3 were their hybrids. This indicated thereby that some crossing do takes place under natural conditions in such plots and subsequently it is probable that some gene blocks might be added to the genetic background of maize populations.

(b) **Genetic consequences**

The introgression of genes in a new population is a natural device for introduction of a new gene or gene block from a relatively distant taxa in an another species background since some of the direct interspecific or intergeneric crosses do not allow it and often result in sterility, abnormal growth and physiological imbalance. Wellhausen *et al.* in collaboration with Mangelsdorf (1951) studied a large number of primitive races of maize collected from Mexico. They have made very useful conclusions from these studies and are of the view that many new varieties and races of maize in Mexico and Central America which now provide considerable bulk of maize germplasm have originated as consequence of the introgression of gene pools of ancient maize types with the exotic types and vice-versa. Anderson and Brown (1952) and Brown and Anderson (1947-1948) have made detailed and exhaustive studies about the northern flints and southern dents and on the origin of U.S. cornbelt maize. They have traced the origin of present cornbelt maize to the introgression of southern dents with northern flints. They have supported this fact with a number of cytological, morphological, genetical and historical evidences.

Genetically speaking, the introgressive hybridization brings in a gradual sifting of gene combinations in a population. Since such gene complexes survive under natural conditions, focuses that such introgressed genes bring in better adaptability to the native habitat than either one of the parents, so it is frequently and favourably selected. But it does not necessarily mean that all the gene introgression is desirable or expresses a heterotic advantage. What apparently is clear is that it brings in new genes in a population and increases its genetic content and gene diversification which could be tapped for productive breeding research. The present day U.S. cornbelt maize is one such good example of the meaningful introgression in economically important crop plants.

(iii) **Evaluation of genetic diversity**

The evaluation of genetic diversity has been of considerable importance in

heterosis breeding programmes. It is primarily from point of view of identifying suitable parental populations for marshalling them into hybrid combinations. Usually in most of the conventional heterosis breeding programmes, geographical diversity or at times, phenotypic diversity is taken as the criteria for choosing genetically divergent population for the isolation of inbred lines.

### (A) Genetic diversity and its relation with geographic and phenotypic diversity

Vavilov (1951) while determining the centres of origin of cultivated plants emphasised that the accumulation of genetic variability was one of the major criteria in fixing the centres of origin. The geographical centres of origin of a particular plant species therefore, are generally supposed to have a high degree of genetic diversity. Athwal (1965) emphasised that generally speaking, the varieties from different geographic area should represent a range of genetic diversity. This fact has been further supported by the investigations of Dhawan and Singh (1961) Tobgy *et al.* (1962), Ahloowalia and Dhawan (1963) and Rao and Sriramulu (1964). The point is however, controversial. Moll *et al.* (1962) working in maize made a number of crosses between the varieties obtained from the various geographical areas but could not find direct relationship between the geographical distribution and genetic diversity. Murty *et al.* (1965) have reported that geographical distribution and genetic diversity could not possibly be directly correlated in crops like brown sarson, linseed, wheat, *Nicotiana rustica* and sorghum.

Phenotypic divergence (Plates 6.1 and 6.2) in a population has also been often considered as an index and criteria of genetic diversity. Hayes and Olson (1919) and Wellhausen (1965) while summarizing the results of $F_1$ crosses have indicated that Flint × Dent crosses in maize generally gave higher performance than Dent × Dent or Flint × Flint crosses. In linseed, ecogeographical divergence has been observed to be more or less correlated or running parallel with genetic diversity (Singh 1963). However, true under certain situations, broad generalization in this connection could be rather misleading. Timothy (1963) observed considerable phenotypic divergence between Mexican, Brazilian and Andean maize collections but met with little genetic diversity as expressed in the heterosis of their $F_1$ crosses. He could not obtain the expected extent of heterosis between the crosses of these populations.

#### Multivariate analysis

The quantitative assessment of genetic divergence has started recently utilizing the Mahalanobis $D^2$ technique based on the multivariates. It has been observed to be a good method of grouping or classifying a number of genetic stocks into various genetically diverse groups or clusters and making meaningful interpretations about the genetic divergence in the germplasm collections. The details of this technique and its application to evaluate the genetic diversity has been given by Rao (1952) and has successfully been used by Murty and Pavate (1962), Chandrasekharaiah (1964), Murty (1965), Murty and Anand (1965), Murty and Anand (1967), Murty and Tiwari (1967), and Peter and Rai (1976).

Multivariate analysis is a second degree statistics analysis and is based on a number of characters taken at a time. To obtain the useful informations from this, first the analysis of variance is obtained for all the characters under consideration. Then the variance-covariance (environmental) matrixes are tested by Wilks criterian to test whether there is significant correlation or not between the variables (correlated variables). Normally no correlation is expected between environmental portion of the variability unless these variables themselves are correlated. All the correlated variables (X's) are then transformed to uncorrelated variables (Y) by the pivotal condensation method as described by Rao (1952). Then from these uncorrelated variables $D^2$ values are calculated by taking the sum of square of the differences between the pairs of corresponding Y values for any of the two populations. Thereafter the grouping of the populations is done depending upon the generalized distance ($D^2$ values) between the various populations. The whole information is then presented by putting them in a diagram (Fig. 6.1). In the diagram, the various populations are so grouped that the populations within the clusters have smaller $D^2$ values than that between the clusters.

**Some additional considerations in the evaluation of genetic diversity**

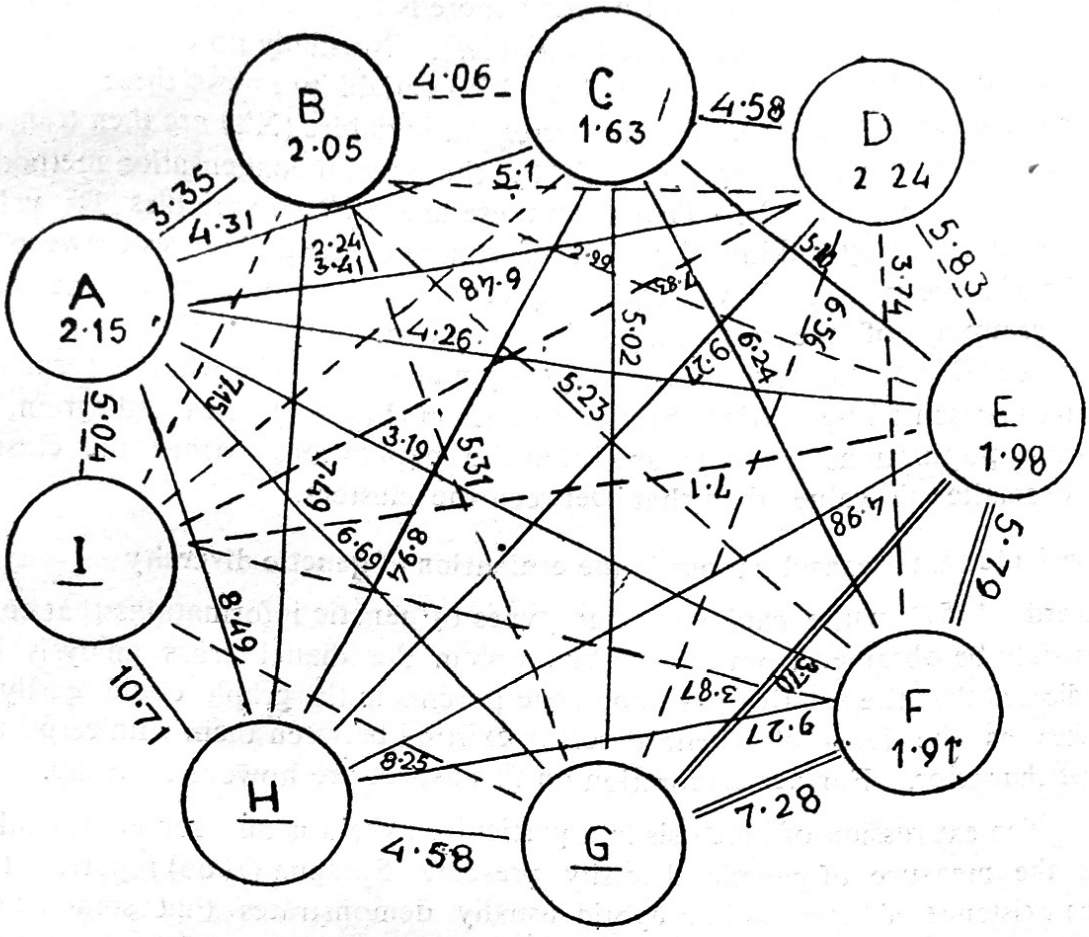
Allard (1957) while explaining the types of genetic informations that could possibly be obtained from the Wr-Vr graph in the diallel cross analysis has indicated that the relative position of the parents in the graph could easily be taken as the level of genetic diversity existing between them with respect to that character. Further elaboration on this aspect are however, lacking.

The expression of hetetosis in a particular hybrid is also sometimes taken as the measure of genetic diversity present. Sprague (1966) reported that the existence of heterosis in a hybrid usually demonstrates that some degree of genetic diversity is existing. This, however, is taken as rather a confirmatory test in evaluating the existence of genetic diversity present in the parents in a crossing programme.

**(iv) Role of genetic diversity in the expression of heterosis**

It has been a general observation that the genetic diversity plays a major role in the expression of heterosis. Nilsson-Leissner (1927) as quoted by Hayes (1963) reported the usefulness of utilizing inbred lines obtained from diverse sources in the expression of heterosis. They utilized three dent and four flint sources for the extraction of inbred lines and made crosses between the lines obtained from same group and also between different groups. The expression of heterosis was more when the crosses were made between various groups than those between the lines obtained from the same source from both dent as well as the flint populations. Wu (1939), Hayes and Johnson (1939), Johnson and Hayes (1940) and Eckhardt and Bryan (1930) presented conclusive evidences to show that the genetic diversity, that is, the source of origin of the inbreds play an important role in the yielding ability of the hybrids. Andres and Bascialli (1940) working in maize have demonstrated the

importance of genetic diversity. They compared the yielding ability of $F_1$ crosses between the inbred lines obtained from the North American and Argentinian sources. The data has been given in Table 6.2.




CLUSTER VARIETIES

| | | | |
|---|---|---|---|
| A | (1, 10, 16, 21, 24, 18) | F | (9, 22, 13, 23) |
| B | (2, 6, 17) | G | (12) |
| C | (3, 4, 11) | H | (15) |
| D | (5, 8, 19, 25) | I | (20) |
| E | (7, 14) | | |

*Fig. 6.1* : Generalized statistical distance among 25 varieties of tomato utilizing $D^2$ statistics (After Peter 1975).

The varieties were : (1) K.T.1, (2) Peru II, (3) 2152-13, (4) 2152-14, (5) S12, (6) Mobaci, (7) B2247, (8) K. Kuber, (9) S1, (10) Pusa Ruby, (11) Sanmarzano, (12) Roma, (13) 63-VF21, (14) Angurlata, (15) Y. Plum, (16) Momor, (17) SL-120, (18) Marglobe, (19) Pusa early dwarf, (20) 1959-53, (21) EPH-7, (22) S-first, (23) Pantnagar local, (24) Best of All and (25) HS-101.

More or less parallel observations have been made by Ahloowalia and Dhawan (1963) with respect to Indian × Indian, American × American and Indian × American crosses in maize.

TABLE 6.2

**Performance of $F_1$ hybrids (After Andres and Bascialli 1940)**

| *Origin* | *No of crosses* | *Kgs/ha* | |
|---|---|---|---|
| | | *Highest cross* | *Average* |
| North American | 15 | 5155 | 3871 |
| Argentine | 48 | 5566 | 3618 |
| North American × Argentine | 34 | 5694 | 4317 |

Shull (1948) while discussing heterosis observed that "The physiological vigour of an organism as manifested in its rapid growth, height and general robustness is positively correlated with the degree of dissimilarities in the gametes by whose union the organism was formed. The more numerous the differences between the uniting gametes, at least within certain limits, the greater on the whole, is the amount of stimulation." Shull's statement clearly showed that the idea of genetic diversity as a mechanism leading to expression of vigour of the hybrids was inherent in his preposition for the term heterosis.

Griffing and Lindstrom (1954) in their studies with cycled inbreds obtained from Brazilian and exotic maize sources with three crosses each within the group and 9 crosses each between the group, however, concluded that heterosis is related to some extent to genetic diversity in these crosses.

Ballosteros (1956) studied the effect of genetic diversity on the expression of heterosis in maize. He reported that the crosses between the inbreds of highest diversity, in general, gave significantly higher yield than those crosses which involved lines from the same parentage or of close origin. The differences between Ist group (highest genetic diversity) and the 3rd group (least genetic diversity) were 11.60 ± 3.04 in favour of Ist group. This was highly significant value. Similarly the differences between the second and the 3rd group were 12.60 ± 3.20 in the favour of 2nd group (intermediate genetic diversity). This value was also highly significant. These observations clearly show that the higher yielding maize hybrids could be obtained by crossing inbred lines extracted from the genetically unrelated populations.

Moll *et al.* (1962) studied heterosis using two varieties obtained from 4 areas that is, south and eastern U.S.A. mid western U.S.A., Puerto-Rico and southern Mexico in all possible combinations. They have reported that the extent of heterosis increases with the increase in genetic diversity within a certain range of divergence. The extremely divergent crosses resulted in decrease heterosis. Gomez (1966) studied heterosis on the varieties utilized

by Moll *et al.* (1962) and made almost similar observations. He reported that the yield increased with increase in genetic diversity upto Nebraska × Puerto-Rico level and then decreased with further increasing in diversity.

### (v) Contributions of various plant characters to genetic divergence

Murty and Arunachalam (1965) have widely reviewed and presented a summarised account of the contributions of various plant characters to the genetic divergence in a number of economically important crop plants. The same has been condensed in Table 6.3.

TABLE 6.3

**Contribution of various plant characteristics to genetic divergence**

| *Crop species* | *Plant characteristics* | *Percent contribution to genetic divergence* |
|---|---|---|
| 1. Wheat | Flowering time | 36.9 |
| | Height of the plant | 27.5 |
| | Grain density | 13.0 |
| 2. Sorghum | Number of spikelets | 68.5 |
| 3. Brassica | | |
| a. *Self incompatible group* | | |
| | Flowering time | 53.4 |
| | Height of the plant | 17.4 |
| | Number of secondary branches | 11.1 |
| b. *Self compatible group* | | |
| | Flowering time | 12.6 |
| | Height of the plant | 32.8 |
| | Number of secondary branches | 12.8 |
| | Number of seeds per siliqua | 22.7 |
| 4. Linseed | Number of primary branches | 78.4 |

### (vi) Morphological characters and their association with specific combining ability

Some of morphological characters have been observed to be good indicators of the specific combining ability in certain of the parental populations. In maize, Anderson and Brown (1952) have reported that a group of carefully choosen characters of typical dent and flint lines could be good criteria in selecting for specific combining ability. They emphasized that the success of utilizing such a method would, however, depend on choosing a

number of morphological, easily scorable attributes which contributes to the genetic divergence either on *a priori* information or based on the experimental evidences. They scored a number of maize inbreds for their number of kernel rows per ear, kernel length, denting expression, husk leaves, number of secondary tassel branches, spikelet length and the number of chromosomal knobs. All the lines were classified and scored with respect to these attributes. An example of the three inbreds Hy, Oh, 40b and Myl has been given in Table 6.4. After scoring the inbreds for these characters an integrated inbred index was obtained for each of them based on these characters.

TABLE 6.4

**Inbred indices based on 7 characters for 3 inbred lines in maize (After Anderson and Brown 1952)**

| *Inbreds* | *Kernel row number* | *Kernel length mm* | *Denting* | *Husk leaves* | *Tassel branches* | *Spikelet length* | *Chromosomal knobs* | *Inbred Index* |
|---|---|---|---|---|---|---|---|---|
| Hy | 9 | 14 | 4 | 14 | 5 | 6 | 12 | = 64 |
| Oh 40b | 2 | 8 | 4 | 1 | 4 | 1 | 3 | = 23 |
| Myl | 14 | 11 | 14 | 14 | 14 | 6 | 9 | = 82 |

Then an indices difference value was obtained for the various inbred combinations (Table 6.5). They selected a number of inbred combinations in descending order of the index differences and actually obtained their $F_1$ yields. The indices differences and the actual yield of $F_1$ crosses were correlated. The r value was observed to be positive and significant during 1948 and its relative magnitude was +0.38** (based on 66 hybrids). During 1949, it was +0.40** (based on 100 single crosses). Emphasising the practical usefulness of this prosedure in heterosis breeding programme, they concluded that by this method it could be possible to easily eliminate 33% of the poor yielding hybrid combinations without loosing the top 10% of the high yielding ones in such a breeding programme.

TABLE 6.5

**Indices differences of the inbred combinations**

| *Inbreds* | *Inbred indices* | *Indices differences* |
|---|---|---|
| Hy | 64 | 41 |
| Oh 40b | 23 | 59 18 |
| Myl | 82 | |

SECTION THREE

# ESTIMTAION OF THE EXTENT OF HETEROSIS

SIX TOPS THREE

# ESTIMATION OF THE EXTENT OF HETEROSIS

7

# Survey of the Magnitude of Heterosis In Various Crop Plants

The information about the extent of heterosis is an important parameter to be assessed in the heterosis breeding programmes. Varying degree of heterobeltiosis with respect to grain yield and other characters have been observed and reported by a number of workers. A condensed account of the extent of yield heterobeltiosis has been given below in Table 7.1 and that of promising crosses have been given in Table 7.2.

TABLE 7.1

**The magnitude of yield heterosis in various crop plants**

| *Sl. No.* | *Crops* | *Heterosis* (Percentage)* | *Characters* | *Authors* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Bajra | 32.5—69.8 | Grain Yield | Ahluwalia and Patnaik (1963) |
| | | Marked | ,, | Tyagi *et al* (1975) |
| 2. | Barley | 20 —50 | ,, | Suneson and Riddle (1944), Suneson (1962) |
| | | 8.3 | ,, | Pawlish and VanVijk (1965) |
| | | 9.1 | ,, | Upadhyay and Rasmusson (1970) |
| | | 26 —47 | ,, | Gebrikidan and Rasmusson (1970) |
| | | 33 —33.5 | ,, | Chaudhary and Singh (1977) |
| 3. | Black gram | 14.9 | ,, | Singh and Singh (1971) |
| 4. | Brinjal | Marked | Fruit Yield | Nagai and Kida (1926) |
| | | ,, | ,, | Pal and Singh (1946) |
| | | ,, | ,, | Venkatraman (1946) |
| | | ,, | ,, | Mishra (1961) |
| | | ,, | ,, | Sambandam (1962) |
| | | ,, | ,, | Baha-Eldin *et al* (1969) |
| | | , | ,, | Peter and Singh (1976) |

TABLE 7.2

**Some of the promising heterotic crosses observed in various crop species**

| *Sl. No.* | *Crop species* | *Heterotic cross combinations* | *Authors* |
|---|---|---|---|
| 1. | Barley | (AB-12159 × EB 1556) | Choudhary and Singh (1977) |
| 2. | Brinjal | (Kalyanpur Type 2 × Pusa purple long) | Peter and Singh (1976) |
| | | ($T_2$ × BGL) | Mishra (1977) |
| 3. | Cotton | (3515 B × M 2608) | Chahal and Singh (1975) |
| | | (SIV 135 × ERB 4492) | Henry and Singh (1976) |
| | | (LSS × A 6476) | Zafar *et al* (1978) |
| 4. | Cowpea | Cross (4 × 7) | Mak and Yap (1977) |
| 5. | Gram | (P 813 × P 1821) | Gowda and Bahl (1976) |
| 6. | Linseed | (B5-33 × EC 1397) | Chandra (1978) |
| 7. | Mustard | (T 46 × KB-1) | Labana *et al* (1975) |
| 8. | Rapeseed | (Arlo × TS 29) | Doloi (1977) |
| 9. | Rice | (SR 26 (B) × Dahar Nagra) | Mallik *et al* (1978) |
| 10. | Wheat | (Sharbati Sonora × WG 450) | Randhawa and Biswas (1977) |
| | | (Sonalika × Kalyan Sona) | Sharma and Singh (1978) |

**Magnitude of heterosis needed for the commercial utilization of the hybrids**

The demonstration of heterosis in a crop species is not adequate justification for the establishment of a commercial programme to produce the hybrids. It has to be carefully evaluated and assessed from the economic feasibility point of view. Usually the acceptance of the hybrid seed by the farmers depends upon the relative cost of the seed and the extra economic gain obtained through the enhancement of the yield by the use of the hybrid over the cultivated variety. Therefore, the assessment of standard heterosis (*i.e.* over the local variety or the check) required to pay the dividends and the investment involved in the hybrid seed costs is perhaps the most plausible approach to assess the possibilities of the hybrid seed utilization. Depending upon the yield level and the nature of the commercial product of the crop, usually a margin of 20-50 per cent of the standard heterosis seems to be reasonable limit. In a crop like wheat, where the initial yield levels are generally good, Reitz (Report on Wheat Quality Conference USDA 1965) and Rodrigues *et al* (1967) have reported that usually a 20 per cent limit is good enough for the commercial utilization of the hybrid seed (Table 7.3). This could be usually a case with other crops also where the level of yield in the hybrids are usually parallel.

For the crops with lower level of production, the amount of heterosis needed may be comparatively high and varying according to the level of production, the relative investment in the purchase of hybrid seed and the relative cost of the produce in the market.

TABLE 7.3

**The percentage of heterosis necessary to pay the additional cost of hybrid seed at different commercial yield leve ls***

| *Commercial yield (q/acre)* | *Additional** seed costs per acre (in rupees)* | | | | | |
|---|---|---|---|---|---|---|
| | *Rs. 30.0* | *Rs. 37.5* | *Rs. 45.0* | *Rs. 60.0* | *Rs. 75.0* | *Rs. 112.5* |
| 2.72 | *20.0**** | *25.0* | 30.0 | 40.0 | 50.0 | 75.0 |
| 4.08 | 13.3 | 16·5 | *20.0* | 26.7 | 33.3 | 50.0 |
| 5.44 | 10.0 | 12.5 | 15.0 | *20.0* | 25.0 | 37.5 |
| 6.59 | 8.0 | 10.0 | 12.0 | 16.0 | *20.0* | 30.0 |
| 8.16 | 6.7 | 8.3 | 10.0 | 13.3 | 16.7 | 25.0 |
| 9.52 | 5.7 | 7.1 | 8.6 | 11.4 | 14.3 | *21.0* |
| 10.88 | 5.0 | 6.3 | 7.5 | 10.0 | 12.5 | 18.8 |
| 12.24 | 4.4 | 5.5 | 6.7 | 8.9 | 11.1 | 16.7 |
| 13.60 | 4.0 | 5.0 | 6.0 | 8.0 | 10.0 | 15.0 |
| 16.32 | 3.3 | 4.2 | 5.0 | 6.7 | 8.3 | 12.5 |
| 19.04 | 2.9 | 3.6 | 4.3 | 5.7 | 7.4 | 10.7 |
| 21.76 | 2.5 | 3.1 | 3.8 | 5.0 | 6.3 | 9.4 |
| 24.48 | 2.2 | 2.8 | 3.3 | 4.4 | 5.6 | 8.3 |
| 27.20 | 2.0 | 2.5 | 3.0 | 4.0 | 5.0 | 7.5 |
| 32.64 | 1.7 | 2.1 | 2.5 | 3.3 | 4.2 | 6.3 |
| 39.80 | 1.3 | 1.7 | 2.0 | 2.7 | 3.3 | 5.0 |

**Adapted from the calculations of Reitz (1965) as extended by Rodriguez et. al. (1967). The values adapted in quintal and rupees per acre taking 1 quintal =3.6743 bushels and 1 dollar = 7.50 rupees.*

*** Refers only to the additional cost of hybrid seed above the amount spent on locally grown variety seed.*

**** Values are the percentage of standard heterosis required when commercial grain value is calculated at Rs. 15/- per bushel or Rupees 55.1145 per quintal.*

SECTION FOUR

# UTILIZATION OF HETEROSIS

# 8

# Breeding Hybrid Varieties

Hybrid varieties are the first generation progeny obtained from a cross involving two parents (inbreds, open pollinated varieties etc.). Here, it is usually used to designate the $F_1$ populations which are produced for the commercial plantings. Some of the hybrids which are commonly used for the commercial production in India are the double crosses (as in maize), double top crosses (as in maize), single crosses (as in sorghum, bajra, cotton, onion etc.). The development of these hybrids have been given as follows :

## A. Development of double cross maize hybrids

The development of double cross hybrids in maize is a multistepped operation. The various steps involved are - (i) collection and evaluation of local and exotic germplasm, (ii) development of inbred lines, (iii) the evaluation of inbred lines by topcrossing, (iv) making single crosses, (v) predicting the yields of double crosses from the single crosses yield data, & (vi) testing the experimental hybrids alongwith checks over years and locations and finally releasing them for the commercial cultivation. The details of the steps involved are as follows :

### (i) Collection and evaluation of germplasm

The first step towards a sound breeding programme is the collection of genetically diverse germplasm. In hybrid breeding programmes, it is more so and includes the collection of genetically broadbased open pollinated varieties, synthetic or composite populations, interracial or intervarietal blends, germplasm complexes, recurrently selected populations etc. It has been adequately emphasized and already given in Chapter-6 that for a higher expression of heterosis, selection of inbred line, from the genetically diverse or unrelated sources is essential.

### (ii) Development of inbred lines

Basically two types of approaches are utilized for the development of inbred lines in maize - (a) the conventional method and (b) the non-conventional methods like the use of monoploids.

### (a) Conventional method

Inbred lines conventionally are developed from the genetically broadbased

populations like open pollinated varieties, composites, synthetics, germplasm complexes etc. by self pollination followed by selection. Initially the plants to be self pollinated for the production of inbreds are selected for their vigour, leafiness, plant height, standing ability, freedom from diseases, pests, good pollen producing capability etc. Then they are selfed and are subsequently grown next year in ear to row progenies. As a consequence of inbreeding usually segregation occurs. During the period of crop growth, as far as possible the plants are subjected to many adversities such as-lodging, diseases, drought, heat or insect infestation. This provides an opportunity to discard the poor plants. Selection is then practiced at the harvest time. The weak and abnormal plants are discarded. Seeds from the desirable plants with a minimum of 25-30 plants are bulked and used for further self-pollination. The self pollination and selection continues till the inbred lines become fixed or true breeding. This usually takes 5-7 generations. At this level, the desirable characters are fixed in practically homozygous state. At this level, the inbreds have a fixed gene frequency which could be easily maintained and reproduced. It is usually believed that 5-7 generations of selfing and selection is desirable when dominance effects are predominant in the expression of economic traits. This usually ensures the maximum performance of the population at the hybrid level. However, in some of the commercial hybrid breeding programmes, a common practice has been to use the inbred lines after 4th or later generations. This is primarily done to save time and to obtain quick results.

In the developmental history of inbred lines, the pedigree of a line is usually indicated by the name of the variety from which developed, station identification, family number and the history of breeding. For example the pedigree of inbred line A Theo-21(B)-F-M-3-F-H / / indicating A Theo as variety name, 21 as family number, B station identification (here for Hyderabad, A.P. India) and F-H-3-F-H/ / as history of breeding where F represents five generations of sibbing summed together and H for number of generations of sibbing. While utilizing the inbreds for the production of commercial hybrids, the lines are given a closed pedigree with the letters CM (coordinated maize) in Indian Maize Breeding Programme and an identification code number. An example of this is CM 104 for A Theo-21(B)-F-H-3-F-H.

In general development of inbred lines, two aspects which are generally considered important are (a) that initially the number of inbred produced must be large and (b) that they are developed from the genetically diverse cources.

**(b) Non-conventional method : use of monoploids**

In addition to the conventional procedure of producing inbred lines, a quick and time saving method for the derivation of inbred lines by detecting and isolating the naturally occuring haploid plants and doubling their chromosome numbers has been suggested and outlined by Chase (1949), (1952). It has

been usually observed that monoploids occur with a frequency of 0.39 to 1.45 per 1000 plants in many open pollinated varieties. Generally, the occurrence of haploid embryos in a population is dependent upon the extent of parthenogenesis prevailing in it. In maize, usually it occurs one to 1000 progenies (Chase, 1966). Most of these monoploids could be detected and then be exposed to the colchicine treatment or as such they may be grown to maturity. It is a common observation that one out of every 10 such plants could be successfully self-fertilized to give homozygous diploid progenies.

The various steps involved in the development of inbred lines with the use of monoploid plants in maize are (i) identifications of monoploid plants with the help of a suitable seedling marker gene or genes (ii) growing the monoploid seedlings to flowering and self pollinating those that produce viable pollen and finally, establishing inbred lines. The details of the steps involved are as follows :

Step 1. Cross the open pollinated variety, single or double cross or topcross hybrid with pollen obtained from a male parent carrying a dominant marker gene such as one which produces purple plant colour or brown or purple colour plumule or any other such suitable marker gene. Check the ears at the harvest time for the kernels resulting from accidental self or cross pollinations with endosperm markers such as-aleurone colour (purple or red).

Step 2. Discard the kernels not showing the endosperm marker phenotype from the harvested ear. Put the remaining kernels for germination.

Step 3. Check the seedlings for plant marker phenotype. Discard all seedlings showing this phenotype. All purple seedlings are assumed to be diploid.

Step 4. Plant the remaining seedlings not showing the marker gene phenotype that are assumed to be monoploid. Then take a root tip for cytological check up of their chromosome number. Further discard the diploid plants if any. The remaining ones will be cytologically confirmed to the monoploids.

Step 5. Grow the monoploid plants to flowering. A spontaneous doubling of chromosomes in a part of tassel in few plants will produce viable pollen and a spontaneous doubling of chromosomes in a part of ear shoot will result in the formation of viable egg cells. Self such plants producing viable pollen and obtain the self seed.

Step 6. Increase the seed of the self-pollinated plants next year. These would be perfectly homozygous diploid plants. Test these plants in hybrid combinations in the same manner as inbred lines developed from the conventional method.

As the monoploid technique is a drastic form of inbreeding, therefore, about 1 to 3 years may be saved in the derivation of inbred lines from an open

pollinated variety. Chase (1952) has reported the derivation of 50 homozygous dent maize lines and a similar number of sweet maize lines at Iowa (U.S.A.). Many lines developed through this techniques are already in use in commercial hybrids. A report indicates that Dekalb Hybrid seed Company Ill. U.S.A. utilized a total of 140 inbred lines to produce hybrids under field trials during 1964. Out of this 60 per cent of lines were developed by Dekalb group and the remaining were obtained from other agencies. Of the 60 per cent Dekalb lines, 25 per cent were monoploid derivatives and 35 per cent were the product of conventional breeding procedure (Chase, 1966). This shows the extent of utilization of the monoploid techniques to the hybrid breeding programme in maize.

### (iii) Evaluation of inbred lines

The next step after the development of inbred lines is to screen out the best performing ones and eliminate those of the low genetic worth. This is initially done by testing the inbreds for their general combining ability prior to testing them for specific combining ability and their use in the commercial hybrids.

The conventional method for the initial screening of the lines is topcrossing. This is perhaps the easiest, economical and quick method of testing a large number of lines for their general combining ability. Davis (1927) for the first time suggested the use of this technique for large scale screening of inbred lines. Jenkins and Brunson (1932) presented the comprehensive data related to the validity of this method. They compared the ranking of the inbred lines as determined by their performance in topcrosses with that of the average performance of the same lines in a number of single crosses with an average of the crosses obtained from 9 to 12 inbreds. The yield obtained from the two types of crosses were then correlated. The correlation coefficient (r) values for yield in two types of crosses were positive and significant and varied from +0.53 to 0.90. In another extensive study, the extent of correlation coefficient obtained in these two sets of values, the r varied from + 0.62 to 6.80. It was, however, generally observed that the lines that gave low yield in topcrosses also produced low yielding single crosses. They concluded that it was safe to discard the lower ranking lines without the risk of loosing the valuable inbreds.

Topcross testing is now greatly utilized and is almost a thoroughfare in the hybrid breeding programmes. This is primarily because with this method it is possible to identify comparatively more number of inbred lines from a group of the size n with only n crosses, instead of all possible $n(n-1)/2$ crosses as is usually the case with single crosses. Numerically, with 50 lines, topcross test will involve making and evaluating 50 crosses only. While with single cross testing it would involve a total of $50 \times 49/2 = 1225$ crosses. This is comparatively almost 24 times more number of crosses than the former one.

The general procedure of topcross testing in maize is that the two lines are alternatively grown with one line of the tester parent (that is an established

open pollinated variety, synthetic or any other heterozygous genetic source). The variety used as tester parent should be low in yielding potential and usually carries the recessive factors for characters of importance so that it could be possible to screen the inbred lines carrying the dominant gene or genes for these characters. Crosses are usually made keeping the inbred as the female parent. The crossed seed is collected and is called topcrossed seed. It is grown the following crop season in a replicated yield trial. The data is statistically analysed and the ranking of the inbred lines is determined with respect to their performance in the topcross trial. Average topcross performance of the lines is worked out. Those lines, which performs better than this over all average in crosses, are generally called good combining lines and those performing less than this average are usually called poor combining lines. From this ranking 10 to 15 upper most ranking lines depending upon the facilities for crossing work available are selected. Such selected lines are generally called 'cream lines'.

**(iv) Making single crosses**

After a number of cream lines have been selected, the next step is making of all possible single crosses between them. For actually making them as (A×B), (A×C), (A×D) etc. usually two lines (10 meters) of A is grown side by side with B, C, D etc. in the order of AB, AC, AD etc. and the crosses are made between them. The crossed seed is collected and the same is planted the following crop season in a replicated yield trial. Yield and other agronomical data is recorded and the same is statistically analysed. The average performance of the single crosses is worked out. From this data, the performance of the double crosses is theoretically predicted.

**(v) Predicting the yields of double crosses from single crosses yield data**

Prediction of the yield of double crosses from single crosses yield data eliminates the field testings of a large number of all possible double crosses. The number of all possible double crosses that could be made from n number of lines (excluding receprocals) can be obtained by the formula :

$$\frac{3n\ (n-1)\ (n-2)\ (n-3)}{24}$$

For 10 inbred lines, 630 double crosses could be made and from 20 the number may be 14,535. The task of breeder in making all these double crosses in field would be enormous, rather almost impossible. It would thus necessitate that an efficient way may be found out for the prediction or the assessment of performance of all possible double crosses theoretically.

For the first time, Richey and Sprague (1931) attempted and suggested a method of predicting the yield of double crosses but could not obtain encouraging results. Three years later, however, Jenkins (1934) provided a convincing basis for the prediction of the performance of the double crosses

from the single cross yield data. He actually correlated the actual yield of 42 double crosses obtained from the replicated yield trial with the predictions based on 4 methods which were serially.

1. Average yield of 6 all single crosses, *i.e.* $(A\times B)\times(C\times D) = \frac{(A\times B)+(A\times C)+(A\times D)+(B\times C)+(B\times D)+(C\times D)}{6}$ among a set of 4 inbreds A, B, C and D.

2. Average yield of 4 non-parental single crosses *i.e.*, the yield of double cross $= (A\times B)\times(C\times D) = \frac{(A\times C)+(A\times D)+(B\times C)+(B\times D)}{4}$

3. Average yield of 4 inbred lines in all single cross combinations *i.e.*, $(A\times B)\times(C\times D)$ = Average of the mean yield of each of the 4 inbreds in a number of single crosses.

4. Average topcross yield of the set of four inbred lines *i.e.* $(A\times B)\times(C\times D) = \frac{(A\times OP)+(B\times OP)+(C\times OP)+(D\times OP)}{4}$

The correlation coefficients between the actual and the predicted values in all cases were positive and highly significant. These were numerically 0.75, 0.76, 0.73 and 0.61 respectively for the methods 1, 2, 3 and 4 as enumerated above.

It was observed that method 2 *i.e.*, the prediction based on the average yield of 4 non-parental single crosses is the best one. Genetically speaking, this method permit the recognition of non-additive gene effects arriving from the dominance or epistasis *i.e.*, $\sigma D^2+\sigma AD^2+\sigma DD^2$ or in other words the specific combining ability. But usually the predictions based on the methods 1, 3 and 4 give primary emphasis on the additive gene action *i.e.*, $\sigma A^2+\sigma AA^2+\sigma AAA^2$. These also assume that order of pairing has little or no importance in determining the over all yield of the double cross hybrids.

The experimental results obtained by Anderson (1938) indicated the superiority of the method 2 in the prediction of the performance of double cross hybrids. Doxtator and Johnson (1936) and Eckhardt and Bryan (1940) have reported the order of arrangement of lines in a double cross had a significant effect on the yield and productivity of the double cross hybrid, especially, when the inbred lines to be used in a double cross are of diverse origin. They reported that if inbred lines A and B are obtained from one source and X and Y from the other, then a uniformly maturing high yielding double cross would likely be obtained by the double cross $(A\times B)\times(X\times Y)$ than that of the $(A\times X)\times(B\times Y)$. Pinnell (1943), however reported that no such effect exists.

The data provided by Anderson (1938) convincingly confirmed the accuracy of the method 2. The actual yield of 10 single crosses as obtained by him from the all possible crossing of 5 inbreds line numbers 23, 24, 26, 27 and 28 given here as A for 23, B for 24, C for 26, D for 27, and E for 28 for sake of simplicity has been given in Table 8.1.

TABLE 8.1

**Yield of 10 all possible single crosses among 5 inbred lines of maize (After Anderson, 1938)***

| *S. No.* | *Crosses* | *Yield (q/ha)* |
|---|---|---|
| 1. | (A × B) | 27.9 |
| 2. | (A × C) | 42.0 |
| 3. | (A × D) | 47.5 |
| 4. | (A × E) | 43.2 |
| 5. | (B × C) | 44.0 |
| 6. | (B × D) | 48.3 |
| 7. | (B × E) | 45.9 |
| 8. | (C × D) | 43.0 |
| 9. | (C × E) | 40.5 |
| 10. | (D × E) | 39.9 |

* *Values adapted in Quintals per hectare.*

From these 5 lines, $(5\times3) \times 4\times3\times2/24 = 15$ double crosses are possible (Table 8.2).

TABLE 8.2

**Percentage of all possible double crosses that could be obtained from 5 inbred lines**

| *Inbred lines* | *All possible single crosses* | *All possible combinations of 4 inbreds* | *Percentage of double crosses* |
|---|---|---|---|
| *(5)* | *(10)* | *(5)* | *(15)* |
| A | (A × B) | A B C D | (A × B) × (C × D) |
| B | (A × C) | | (A × C) × (B × D) |
| C | (A × D) | | (A × D) × (B × C) |
| D | (A × E) | B C D E | (B × C) × (D × E) |
| E | (B × C) | | (B × D) × (C × E) |
| | (B × D) | | (B × E) × (C × D) |
| | (B × E) | C D B A | (C × D) × (A × E) |

| (5) | (10) | (5) | (15) |
|---|---|---|---|
| | (C × D) | | (A × C) × (D × E) |
| | (C × E) | | (C × E) × (A × D) |
| | (D × E) | D E A B | (D × E) × (A × B) |
| | | | (A × D) × (B × E) |
| | | | (B × D) × (A × E) |
| | | E A B C | (A × E) × (B × C) |
| | | | (A × B) × (C × E) |
| | | | (A × C) × (B × E) |

The method of the prediction of the performance of the first three double crosses (A×B) × (C×D), (A×C) × (B×D) and (A×D) × (B×C) has been given in Table 8.3.

TABLE 8.3

**Methods of predicting yield of three different double crosses (A×B) × (C×D), (A×C) × (B×D) and (A×D) × (B×C) that could be made from 4 inbred lines (A, B, C and D) with the use of yields (q/ha) of all possible single crosses (Table 8.1) (After Anderson, 1938)***

| S. No. | Double crosses | Non-parental single crosses | | Predicted yield (q/ha) |
|---|---|---|---|---|
| | | Parentage | Yield in (q/ha) | |
| 1. | (A × B) × (C × D) | (A × C) | 42.0 | |
| | | (A × D) | 47.5 | |
| | | (B × C) | 44.0 | |
| | | (B × D) | 48.3 | |
| | | Average | 45.4 | 45.4 |
| 2 | (A × C) × (B × D) | (A × B) | 27.9 | |
| | | (A × D) | 47.5 | |
| | | (B × C) | 44.0 | |
| | | (C × D) | 43.0 | |
| | | Average | 40.6 | 40.6 |
| 3. | (A × D) × (B × C) | (A × B) | 27.9 | |
| | | (A × C) | 42.0 | |
| | | (B × D) | 48.3 | |
| | | (C × D) | 43.0 | |
| | | Average | 40.3 | 40.3 |

* *Values adapted in quintals per hectare.*

The predicted yield of the 15 double crosses (Table 8.2) have been given in Table 8.4.

TABLE 8.4

**The actual and predicted yields of fifteen double crosses that could be obtained by 10 single crosses as given in Table 8.1 and Table 8.2 (After Anderson, 1938) ***

| *S. No.* | *Double crosses* | *Yield (q/ha)* | |
|---|---|---|---|
| | | *Actual* | *Predicted* |
| 1. | (A × B) × (C × D) | 46.1 | 45.4 |
| 2. | (A × C) × (B × D) | 41.8 | 40.6 |
| 3. | (A × D) × (B × C) | 41.6 | 40.3 |
| 4. | (B × C) × (D × E) | 47.1 | 44.6 |
| 5. | (B × D) × (C × E) | 41.6 | 43.4 |
| 6. | (B × E) × (C × D) | 42.0 | 43.2 |
| 7. | (C × D) × (A × E) | 44.0 | 42.5 |
| 8. | (A × C) × (D × E) | 45.7 | 43.6 |
| 9. | (C × E) × (A × D) | 43.6 | 42.0 |
| 10. | (D × E) × (A × B) | 47.7 | 46.4 |
| 11. | (A × D) × (B × E) | 38.9 | 39.8 |
| 12. | (B × D) × (A × E) | 38.9 | 40.5 |
| 13. | (A × E) × (B × C) | 37.5 | 39.2 |
| 14. | (A × B) × (C × E) | 43.6 | 43.9 |
| 15. | (A × C) × (B × E) | 40.1 | 38.9 |

* *Values adapted in quintal per hectare.*

As is apparent in Table 8.4, the predicted yield of the double cross (A × B) × (C × D) was 45.4 q/ha The actual yield obtained later by the experimental yield trial was observed to be 46.1 q/ha. These value for practical purposes are very close. Hayes *et al.* (1943) compared the value of predicted yield of 8 double crosses with the actual yield obtained where the trials were conducted with 5 replications in each of 4 locations. The difference between the actual and the predicted values obtained were minor and of no great importance.

It is now generally believed that the prediction of the performance of the double crosses would be better estimated when the data of the average yield of single crosses is obtained from more seasons and locations as compared to the one obtained in one year and at one location.

**(vi) Testing experimental hybrids**

After the double cross yield have been predicted, a number of highest yielding double crosses are chosen and these are actually made. These are called

experimental double cross hybrids and are grown in elaborate yield trials over years (about 3) and locations (about 15) to determine their actual performance in the field in comparison with the local check varieties and the best hybrid already under commercial production. The results obtained over years and locations are discussed in national scientific forum and if found suitable are released. A list of the double cross hybrids which have been actually released since 1961 to date in India has been given in table 8.5.

TABLE 8.5

**Characteristic features of certain double cross maize hybrids released in India**

| *S. No.* | *Hybrids* | *Maturity (in days)* | *Yield potential (q/ha)* |
|---|---|---|---|
| 1. | Ganga Hybrid Makka 1 | 80-90 | 40-50 |
| 2. | Ganga Hybrid Makka 101 | 95-105 | 46-60 |
| 3. | Ranjit Hybrid Makka | 100-110 | 50-60 |
| 4. | Deccan Hybrid Makka | 100-110 | 45-65 |
| 5. | VL 54 | 100-110 | 45-65 |
| 6. | Ganga Hybrid Makka 3 | 90- 95 | 45-65 |
| 7. | Himalayan Hybrid Makka 123 | 105-110 | 45-65 |

**B Development of double topcross hybrids in maize**

Double topcrosses are the hybrid progenies between a single cross and an open pollinated variety. The major reasons for the utilization of the double topcrosses are that while utilizing and retaining the hybrid vigour of single cross, it appears possible to utilize the local adaptability, quality considerations, resistance to diseases and pests etc. of the open pollinated varieties in a purposeful crossing and making the hybrid rather better acceptable for commercial cultivation under the conditions and areas where the varieties crossed are grown. Various steps involved in the development of double topcross hybrid are (a) the production of a number of promising single crosses, (b) crossing them with a series of open pollinated varieties, (c) testing their performance in elaborate yield trials over years and locations and finally releasing them for commercial cultivation. In maize breeding programmes, the development of double topcross hybrids could as well be initiated as an offshoot of a double cross breeding programme. A few of best single cross hybrids obtained in the single cross yield trials (as given in step 4 of developing double crosses) could be crossed with a number of promising open pollinated varieties (both, local and exotic) and run into a yield trial along with the standard hybrids and other checks. The best double topcross which significantly exceeds the performance of the recommended hybrids may be picked and recommended for commercial cultivation. The double top cross maize hybrids which have been released for

commercial cultivation in India are Ganga Safed Hybrid Makka 2, Hi-Starch and Ganga Hybrid Makka 5. (Table 8.6)

TABLE 8.6

**Characteristic features of double topcross hybrids Ganga Safed Hybrid Makka 2, Hi-Starch and Ganga Hybrid Makka 5**

| *S. No.* | *Hybrids* | *Maturity (in days)* | *Yield potential (q/ha)* |
|---|---|---|---|
| 1. | Ganga Safed Hybrid Makka 2 | 92-95 | 50-65 |
| 2. | Hi Starch Hybrid Makka | 90-95 | 45-60 |
| 3. | Ganga Hybrid Makka 5 | 90-95 | 45-65 |

**C. Development of single cross sorghum hybrids**

Single cross hybrids are the progenies of a cross between two parent. In crops like sorghum, because of its predominantly self-pollinating nature, there is not much inbreeding depression and loss of vigour. Therefore, the varieties are straight way used for hybrid seed production. These varieties, *per se* unlike maize inbreds, are vigourous and produce enough seed on them. Thus, in this crop, the production of double crosses to obtain hybrid seed is unwarranted and generally single crosses are utilized for commercial hybrid seed production programmes.

In a crop like sorghum where flowers are bisexual, the availability of cytoplasmic male sterility makes the production and utilization of hybrids economically feasible. In this crop, unlike maize, the production of hybrid is a simple operation. Preliminary and final evaluation both involved single crosses with a single male sterile female line. Usually, a large number of single crosses are made with the lines of diverse origin, their performance is evaluated without involving any prediction of performance in the elaborate yield trials. The best combining crosses are then selected for multi-locational testings. Finally using the same male sterile the commercial hybrid seed with the best performing parentage is produced.

The development, maintenance and increase of male sterile lines, in corporation of fertility restorer genes in male parent has been given in Chapter 9 (*i.e.*, commercial exploitation of heterosis).

In India, utilizing CK 60 (combined Kafir 60) a stable, high combining, dwarf, insensitive to day length, resistant to shoot borer, male sterile line, a large number of varieties with yellow or white pearly endosperm were crossed. About 100 crosses were made in 1961. Field trials were conducted through out the country during 1962. By 1965, two hybrids CSH 1 and 2 were released. These hybrids have given 60 - 80 per cent better yield than the local sorghum varieties. Recently two more hybrids viz., CSH 3 and 4 have been added to this list of the hybrids in this crop.

9

# Commercial Exploitation of Heterosis

From the preceeding chapters, it is now clear that the heterotic advantage is limited only to the $F_1$ generation and that for obtaining this advantage, the farmers have to utilize the fresh hybrid seed year after year. It is, then necessary that the hybrid seeds must be produced, processed and distributed commercially on large scale each year. Cheap availability of the hybrid seed is key and crucial and perhaps the most important factor in the usage of the hybrid seed by the farmer. It may be recalled here that though the heterosis in maize was reported as early as 1910, but its commercial exploitation and utilization could not be made possible till Jones (1918) suggested the technique of double crosses to produce the hybrid seed. The only advantage of utilizing double crosses seed over that of the single crosses seed was that while maintaining the yield levels of single crosses, it was possible to deliver the seed at the cheap rates.

The hybrid seed could be produced at cheap rates only if the crop species have certain builtin morphological or physiological mechanisms suitable for the control of parentage for successful hybrid seed production and its floral morphology is amenable to it. Some of the basic requirements and the techniques employed in the large scale commercial hybrid seed production in crop plants are as follows :

*Basic Requirements* :

1. Availability of a proven heterotic hybrid combination which could distinctly and profitably surpass the yield levels of the commercial variety being grown.

2. Availability of an easy, economic and effective means of eliminating or rendering functionlessness of the male part of the bisexual seed parent, mechanically, genetically or even biochemically.

3. Availability of a strong fertility restoration system (in case of the use of cytoplasmically governed male sterility system) or availability of a tightly linked marker gene system in the case of genetically governed male sterility. Full and the detectable expression of self incompatibility.

4. Absence of modifier genes or gene systems in the case of the use of self incompatibility for hybrid seed production.

5. Complete synchronization of flowering in both seed and the pollen parents.

6. Free, unrestricted and natural transfer of pollen from pollen to seed parent.

7. Good seed setting on the seed parent.

8. A skilled organized effort for large scale seed production, certification, processing and well knitted distribution channel of hybrid seeds.

**Types of hybrids used for commercial cultivation**

The various types of hybrids utilized in the hybrid seed production programmes of various crops are the single crosses, double crosses, double top-crosses, triple crosses etc. A summarised account of these hybrids has been given in Table 9.1.

TABLE 9.1

**Commercial hybrids used in some of the crop species**

| *Sl. No.* | *Hybrids* | *Symbolic representation* * | *Crops* |
|---|---|---|---|
| 1. | Single crosses | (A×B) | Bajra, Brinjal, Carrot, Castor, Chillies, Cotton, Cucurbits, Onion, Sorghum, Tobacco, Tomato, Watermelons, Wheat |
| 2. | Double crosses | (A×B)×(C×D) | Maize, Sugarbeets |
| 3. | Threeway crosses | (A×B)×C | Sweet maize. |
| 4. | Double top crosses | (A×B)×OPV | Maize |
| 5. | Triple crosses | (A×B)×C×(D×E)×F | Cabbage, Kale |
| 6. | All types of hybrids | — | Potato, Sugarcane, etc. |

* *A,B,C,D,E and F are constituent inbred lines of the hybrids. OPV is the symbol for the open pollinated variety.*

**Techniques for the production of hybrid seeds**

Preparation of seed parent of the heterotic cross through mechanical or genetic emasculation to receive the pollen of the desired parent is the most important component of the hybrid seed production programmes. Pollination is almost all the times is done by natural agencies though in some cases it is accomplished through the manual operations. The conditioning of the female parent for natural crossing is usually done either by hand removal of the

anthers from the female plants by hand emasculation, mechanical removal of male infloresencence (detasseling) etc., or by the utilization of genetic means such as incorporation of male sterility (cytoplasmic, genetic or both), self incompatibility in the seed parent or may be accomplished through the uses of chemicals *i.e.*, the selective male gametocides etc. A brief description of some of these techniques is as follows :

**(1) Hand emasculation**

The technique of hand emasculation and pollination could be good method for producing hybrid seeds for certain choiced flowers, vegetables or fruits where single pollination produces very large number of seeds and the seed is needed for planting only on a limited scale. It could be and has been successfully employed in toe hybrid seed production of plants like brinjal, watermelons, tomato, tobacco squashes, cucurbits where single pollination produces 500 to 25,000 seeds (Sambandam 1962). In India, this method has also been utilized for the production of hybrid seeds in cotton. The cost of production of hybrid seed by this method under the conditions of cheap availability of labour has been observed to vary from Rs. 3/- to Rs. 21/- per pound (Patel and Patel 1952 and Thakar and Seth 1955). Kaiwar (1957) working with cotton suggested that the labourious process of hand pollination in cotton could be overcomed by taking advantage of the time difference between the opening of the flowers and anthers dehiscence in the cross Laxmi × V 135. The time of dehiscence of the anthers in the variety Laxmi is usually 10.45 a.m. while that in the variety V 135 is 8 a.m. Utilizing hairiness and petal spotting pattern as marker genes, he actually produced the hybrid seed taking advantage of this difference and found that 80% of the crossed seeds were hybrids and 20% were selfs.

For the utilization of the above method, the availability of a genetic marker gene preferably a seedling marker is helpful and is rather a must for the detection and elimination of possible selfs from the crossed ones. Especially marker characters like seedling pigmentation, hairiness or non-hairiness, leave shapes, sizes, cotyledon colours etc. are much helpful in initially eliminating the selfs. This is true and is possible in crops like vegetables where usually transplanting of the seedlings is done and is actually now being followed in the hybrid seed production of brinjal and chillies (Singh 1972). Methods for the utilization of marker genes and the handy emasculation and pollination in watermelon have been reported by Singletary and Moore (1965) and in tobacco by Egerer (1964).

**Detasseling for the preparation of the seed parent in the hybrid seed production in maize**

Detasseling is the process of manual or mechanical removal of the immature tassel before they start shedding their pollen for the preparation of the seed parent in the hybrid seed production. Maize plant especially is much suited for the detasseling as the male inflorescence (*i.e.*, tassel) is situa-

ted at the top of the plant, is in a convenient package and could be destroyed without disturbing the female flowers. The detasseled plants become the seed parent and receive the pollen from the pollen or male parent for the hybridseed production.

For the commercial hybrid seed production in maize usually 4 lines of seed parent are planted alongwith 2 lines of pollen parent side by side and are allowed to grow together (Plate 9.1). The minimum field isolation from one field of seed production is kept at 200 meters. When the plants approach towards the tasseling time, the immature and emerging tassel from the seed parent are mechanically. removed. Thus, the emerging silks on the seed parent receive the pollen from the plants of the male parents. The seed harvested from the seed parent are practically all hybrid seed and this is harvested, processed and is sold as the commercial hybrid seeds.

**Utilization of male sterility for hybrid seed production**

Large scale hybrid seed production sometimes becomes handicapped because of high labour cost, their inadequate supply and at times because of seasonal fluctuations in weather conditions like continuous raining etc., at the time detasseling operations. To cope with such of the problems, the plant breeders in the last 25 years have discovered usable working plans to elevate such difficulties by the utilization of male sterility and self incompatibility mechanisms for the preparation of seed parents for hybrid seed production in a number of crops. Of these, male sterility has been found to be considerably promising and is presently being utilized in the hybrid seed production of onion, sorghum and a large part of the hybrid maize in U.S.A. Almost all the sorghum and bajra hybrids now grown in India are produced by the utilization of this biological phenomenon.

In plants, male sterility occurs primarily because of the production of non-functional or abnormal male gametes and is mainly caused by the chromosomal aberrations, pollen abortion, cytoplasmic × genome interactions or the cytoplastic influences that upset the normal development of the pollen. It may also be because of the failure of anthers dehisence, anther abortion or its pistillody. In plants, the male sterility has been found to be of three types : cytoplasmic, genetic, and cytoplasmic-genetic.

The cytoplasmic male sterility is a type of male sterility which is governed by the factors carried through the cytoplasm or the female line and are not diluted or lost in the successive generation of reproduction. The male sterile plants have sterile cytoplasm while the fertile plants have fertile cytoplasm. The progenies of the male sterile plants are usually male sterile *i.e.*, it is transmitted by the maternal plant. This type of male sterility is maintained by crossing it with a maintainer line containing the male fertile cytoplasm.

Genetic male sterility is a type of male sterilty in which the expression of this character is governed by genetic factors. In a number of crop plants, the male sterility condition of this type has been observed to be governed

originally by homozygous recessive genes (ms ms). In such a type, the male sterile stocks are maintained by crossing male sterile plants with heterozygous fertile plants. Half of the progenis are sterile and the other half are heterozygous fertile as illustrated below :

(1) ms ms × Ms ms
male sterile heterozygous male fertile
ms ms 50% plants male sterile
Ms ms 50% plants heterozygous male fertile

(2) ms ms × Ms Ms
male sterile Homozygous male fertile
Ms ms All heterozygous male fertile

| Ms Ms Ms ms | — | ms ms |
|---|---|---|
| 3/4 male fertile | | 1/4 male sterile |

The expression of male sterility in cytoplasmic genetic type is conditioned by the inter-play of both cytoplasm and the genetic factors *i.e.*, it is conditioned by the action of a specific type of genes put in a particular type of cytoplasm.

It is clear from the above discussion that a plant would be male sterile only if it carries sterile (S) cytoplasm and when both genes are also of sterile type (ms ms). The gene Ms is dominant over gene ms. In this type, the maintainer parent that carries male fertile character carries either genes (Ms Ms or Ms ms) which have the power to restore the fertility or the pollen producing capability even in the sterile cytoplasm. This gene is usually called as the fertility restorer gene.

**Cytoplasmic male sterility and hybrid seed production**

Cytoplasmic male sterility is one of the extensively used type of male sterility in hybrid seed production programmes. The existence of cytoplasmic male sterility in maize was first observed and reported by Rhoades (1931) and was first suggested for its utilization for hybrid maize production by Krug (1932). The proposal of Krug necessitated the production of atleast 1/3 of the hybrid seed to be produced by detasseling and to be blended with 2/3 or the seed produced through the male sterile source without detasseling. The existence of cytoplasmic sterility was later reported in onion, sugarbeet, sorghum, bajra, wheat etc. A summarized account of the various reports on the cystoplasmic male sterility in various crops has been given in Table 9.2.

Cytoplasmic male sterility has been extensively utilized in the production of hybrid seed in onion, maize, sorghum and sugarbeet. The commercial utilization of hybrid seed in sorghum, bajra and wheat became possible only after the availability of the cytoplasmically governed male sterilelines and their ferility restorer systems in these crops. Duvick (1966) has reported that during 1962 in U.S.A. 23% of the acreage grown under onion was hybrid

seed, 60% of the acreage grown under sugarbeet was hybrid sugarbeet, 85% of the acreage grown under maize was produced through the use of cytoplasmic male sterility and 90% of the sorghum hybrid were all produced by this system. Recently the cytoplasmic male sterility system have been employed for the production of bajra and sorghum hybrids in India and is now being extended to the development of hybrid wheat.

TABLE 9.2

**Cytoplasmic male sterility in crop plants**

| *Sl. No* | *Crops* | *Authors* |
|---|---|---|
| 1. | Bajra | Burton (1958), (1965), Burton & Athwal (1967) |
| 2. | Carrots | Welch and Crimball (1947), Thompson (1961), Banga and Van Bennekom (1964) |
| 3. | Chillies | Peterson (1958), Ohta (1961a) (1961b) |
| 4. | Cotton | Meyer and Meyer (1961), (1965), Richmond & Kohel (1961), Sarvella (1964) |
| 5. | Cucumber | Barnes (1961) |
| 6. | Field beans | Bonel, Fycee and Toynbeeclarke (1966) |
| 7. | Maize | Rhoades (1931) (1933), Josephson and Jenkins (1948), Jones (1950), Jones & Mangelsdorf (1951), Rogers and Edwardson (1952), Jones, Stinson & Khoo (1957) |
| 8. | Onion | Jones & Clarke (1943), Jones and Emsweller (1943), Duvick (1959), Yen (1959), Andrasfalvy and Balint (1964) |
| 9. | Rice | Kitamura (1962) |
| 10. | Rye | Cehovskaja (1965) |
| 11. | Sorghum | Stephens and Holland (1954), Maunder and Pickett (1959), Craigmiles (1961) (1962), Hadley and Singh (1961), Joglekar and Deshmukh (1961), Kid (1961) |
| 12. | Sugarbeet | Knapp (1955), Stein *et al* (1959), Cleij and Kloen (1963), Nagovski and Makagon (1963) Bulin and Bulin (1966) |
| 13. | Sunflower | Kinman (1963) |
| 14. | Tabacco | East (1932), Clayton (1950) |
| 15. | Tomato | Rick (1944) (1945), Rick and Butler (1956), Anderson (1963) |
| 16. | Wheat | Kihara (1951) (1962), Fukusawa (1953) (1955) (1958), Kihara & Tsunewaki (1962), Schmidt *et al* (1962), Wilson and Ross (1962), Kherde (1966), Livers (1964), Shebeski (1965), Nettevick and Fedorova (1966), Ochler & Ingold (1966), Oganerjan (1966), Watson & Mcwhirter (1968) |

**Utilization of cytoplasmic male sterility in hybrid maize seed production**

The process of detesseling is labourious and often has been observed to affect the yield (Jones & Mangelsdorf 1951). To elliminate the tedium of this process in maize, the cytoplasmic male sterility has been successfully utilized. This is incorporated basically into the inbred lines that are used as the seed parent in the production of single crosses. The male sterility attribute of donor source is incorporated in the desired line (which does not carry the fertility restorer genes) by introducing the chromosomes of the lines into the sterile cytoplasm by crossing the inbred as the male parent to the male sterile inbred line followed by repeated backcrossing (usually 6 to 7). This new male sterile line (A, the female parent of the single cross) is then maintained by pollinating it by its original fertile counterpart (B)

In maize, cytoplasmic male sterility has been identified from several sources (Duvick 1966). The two major sources are Taxas (T) type and USDA (S) type. A comparative idea of these two types of cytoplasmic male sterility has been given in Table 9.3.

TABLE 9.3

**Differences between S and T sources of cytoplasmic male sterility in maize (After Duvick 1966)**

| *Sl. No.* | *Characteristics* | *S Type* | *T Type* |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| 1. | *General Features* | | |
| (*i*) | *Source known as* | USDA source | Texas source |
| (*ii*) | *Discovered by* | Jones (1944) at Connecticut | Mangelsdorf (1944), Rogers (1944), Rogers & Edwardson (1952) in Mexican June. |
| (*iii*) | *Morphological effect on anthers* | | |
| (a) | *in fully sterile condition* | No difference | No difference |
| (b) | *in partially fertile condition* | Needle like anther appearance | Gnarled anther appearance. |
| 2. | *Genetics of male sterility* | | |
| (*i*) | *Cytoplasmic background* | Different than T | Different than S. |
| (*ii*) | *Reaction with U.S. maize lines.* | Few lines sterile | Most lines sterile |
| (*iii*) | *Fertility restoration* | | |
| (a) | *Normal season* | Dominant gene RF 3 needed | Two complimentary dominant genes RF 1 and RF 2 needed |

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| (b) | *Hot & dry season* | | |
| | (*i*) *Full restoration* | Rf 3 Rf 3 | Rf 1 Rf 1 Rf 2 Rf 2 with dominant modifiers. |
| | (*ii*) *Partial restoration* | Rf 3 Rf 3 | Rf 1 Rf 1 Rf 2 Rf 2 or rf 1 rf 1 Rf 2 Rf 2 with dominant modifiers. |

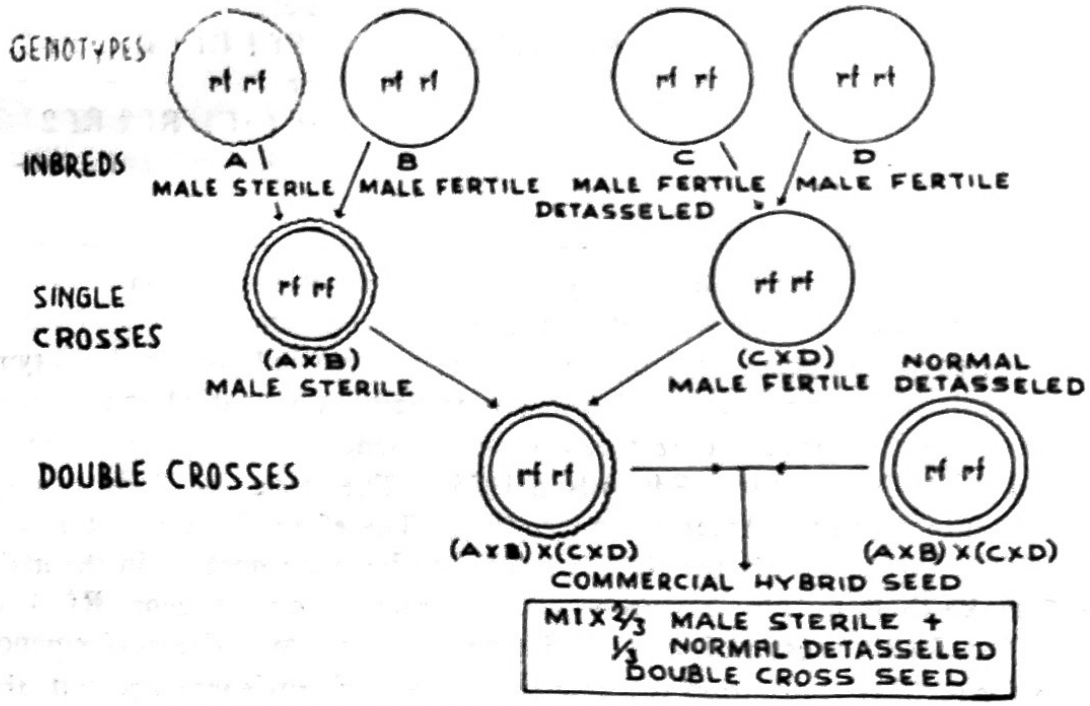
These above mentioned two types of cytoplasmic male sterility differ mostly from their morphological effect on the anthers and the fertility restoring genetic system. When the anthers are partially fertile the S type gives a needle like appearance while the T type bestows a gnarled appearance. In Texas type, the fertility is restored by the presence of two dominant genes Rf 1 and Rf 2 (Duvick 1965, Edwardson 1955). The gene Rf 2 is usually present in almost all the American maize lines. Therefore, for the restoration of fertility in this type only the Rf 1 is needed to be incorporated in the male lines to make them fertility restorers. A separate dominant gene Rf 3 is needed for fertility restoration in the S type of cytoplasm. The performance of Rf 3 for fertility restoration is stable in all types of environments but the Rf 1 and Rf 2 give complete fertility only in the favourable environments. In hot and dry conditions certain modifier genes are also needed for the complete fertility restoration.

The incorporation of a fertility restorer gene into an inbred line may be accomplished by a backcrossing programme preferably by adding it to sterile inbred so that the plants with a dominant restorers may be conveniently identified following each cross without testcrossing it to a male sterile line.

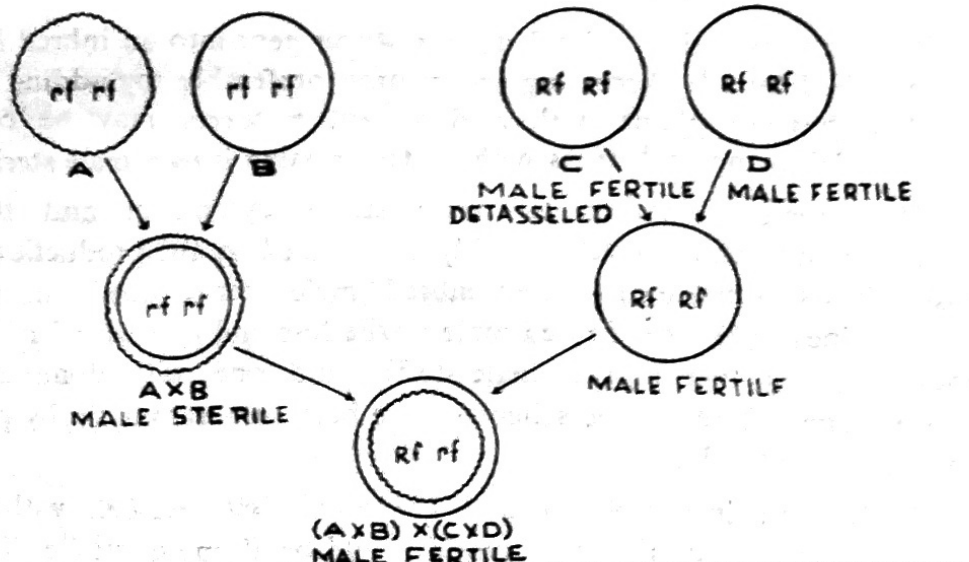
The three combinations of male sterile cytoplasm and the fertility restorer genes which could possibly be utilized in the production of double cross hybrids in maize or (1) one inbred male sterile line with no fertility restorer lines; (2) one inbred male sterile line and two with fertility restorer genes and; (3) two inbred male sterile and one with dominant fertility restorer gene. These three schemes have been diagrammatically presented in Fig. 9.1 (a) and 9.1 (b).

As is given in Fig. 9.1 (a) and (b) in the first case *i.e.*, without fertility restorer gene, the doublecross hybrid would be all male sterile. Therefore, it would be necessary to blend it with atleast 1/3 to 1/2 of the male fertile doublecross seed produced by normal detesseling. It would provide adequate pollination of the doublecross plants in the farmers field. In this case, therefore, the use of male sterility eliminates the detesseling only partially. In the second case *i e.*, with two fertility restorer genes the detesseling process

would be eliminated by half and in the third case *i.e.*, with a dominant fertility restorer gene it completely eliminates the process of detesseling in the field.




SCHEME 1. UTILIZING MALE STERILTY WITHOUT FERTILITY RESTORER GENES(DETASSELING IS PARTIALLY ELIMINATED)

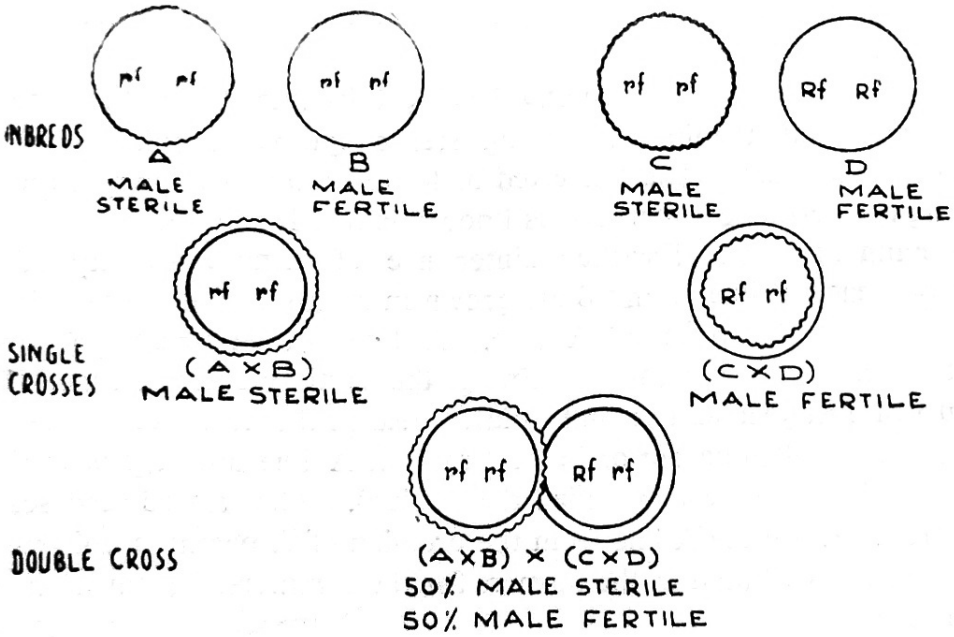



SCHEME 2 ONE MALE STERILE LINE WITH TWO FERTILITY RESTORER GENES

*Fig. 9.1 (a)*: Production of commercial double cross hybrid seed utilizing male sterility (Scheme 1 and 2) (rf = non-fertility restorer gene, Rf = fertility restorer gene)

**Utilization of cytoplasmic male sterility in hybrid sorghum seed production**

Unlike in maize, in sorghum, the single crosses are mainly utilized for the exploitation of heterosis (Table 9.1). The sorghum being a pre-dominantly self pollinated crop, there is hardly any serious inbreeding depression in terms




SCHEME 3. WITH FERTILITY RESTORER GENES
(DETASSELING IS COMPLETELY ELIMINATED)

*Fig. 9.1 (b)* : Production of commercial double cross hybrid seed utilizing male sterility (Scheme 3).

of the loss of size, vigour or productivity and the varieties could be as such used as the inbred lines in maize. The seed could be produced adequately and cheaply on the parental lines themselves. In this crop, the male sterility was first reported to be resulting from the introduction of the Kafir chromosomes into milo cytoplasm. When milo was used as the male parent, the fertility was restored in the male sterile plant. More than two factor pairs are involved in the nuclear portion of the cytoplasm nuclear interaction (Stephens and Holland 1954). One dominant gene $MSc_1$ have been reported to be responsible for the restoration of the fertility. However, in some environments it needs dominant modifiers to give complete fertility restoration.

In U.S.A., a number of varieties with Kafir parentage were converted into male sterile lines by crossing the Kafir variety with milo and by their repeated backcrossing with Kafir as the pollen parent till practically all the genes of Kafir parentage were introduced into milo cytoplasm. One such converted male sterile line now used in the hybrid sorghum breeding programme in India is CK 60 (Combined Kafir 60). This male sterile line has been crossed with a number of Indian and exotic sorghum lines to pick up the best single cross with acceptable grain quality (pearly type) and the one

also containing the requisite fertility restoring gene *i.e.*, $MSc_1$ and the other needed modifier genes.

**Steps involved in the commercial production of hybrid sorghum**

The various steps involved in the commercial production of hybrid sorghum utilizing the male sterility mechanism are the (i) development of the male sterile line; (ii) its maintenance and increase; (iii) production of single cross seed.

The male sterile line is usually developed by introducing the genes of the line or variety into the line containing sterile cytoplasm (like CK 60) by a series of backcrosses. The converted male sterile line is known as line A and the original fertile counterpart is known as line B and latter is usually used as the maintainer line. For the maintenance of the male sterility of line A, two rows each of line A and B are grown in isolation and the seed is collected only from the rows of A line. A line is then crossed with the male parent of the heterotic cross containing the fertility restorer gene. This is then grown next year in a second isolated field in the ratio of 4 rows of A line 2 rows of pollen parent or in 6 : 2 ratio of A line and the pollen parent line (which is otherwise also called the R line). The crossed seed set on the seed parent *i.e.*, on the A line from the crossing of R parent is collected, processed and is distributed to the farmers for the commercial cultivation of the hybrid seed.

Utilizing the male sterile CK 60 source, a number of sorghum hybrids have recently been produced in India utilizing mainly the varieties with white pearly or yellow endosperm which would cross well with it to produce good seed quality in the hybrid and also restores its seed fertility. In this way 100 crosses were made during 1961 and from these, 12 promising hybrids namely, ms CK 60×IS 84, ms CK 60×IS 532, ms CK 60×IS 2930, ms CK 60×IS 2945, ms CK 60×IS 2931, ms CK 60×IS 2932, ms CK 60×IS 3687, ms CK 60×IS 3555, ms CK 60×IS Nandayal, ms CK 60×Aishpuri, ms CK 60×M 35-1 (Rao and House 1965) were selected for further large scale testing during 1963-64 and out of these hybrids two promising ones namely, ms CK 60×IS 84 and ms CK 60×IS 3691 were released by 1965 as CSH 1 and CSH 2.

Similarly in bajra breeding programme in India 256 hybrids were made between a cytoplasmically sterile source MS Tift 23A (Plate 9.2) and a number of Indian and exotic lines. These were tested at six locations during 1963 Kharif and the best of the hybrids exceeded the local checks by 44-232% and consequently as a result of these testings (Plate 9.3) two hybrids namely, HB 1 (Tift 23A×BIL-3B) (Plate 9.4) in 1965 and HB 2 (Tift 23A×J-88) were released in 1966 for commercial cultivation.

**Utilization of male sterility in the production of hybrid wheat**

**Cytoplasmic male sterility in wheat**

The discovery of cytoplasmic male sterility and the pollen fertility restoration

mechanism in wheat has made the production of hybrid wheat a commercial feasibility. Kihara (1951) for the first time reported that *Triticum vulgare* genome when put in the cytoplasm of *Aegilops caudata* gives male sterile plants. During 1958, he selected male sterile *Triticum vulgare* types from hybrid material in which *Triticum vulgare* nucleus was substituted in *A. caudata* cytoplasm. It remained sterile through the successive generations of backcrosses. Later reports of cytoplasmic male sterility in intergeneric combinations of nucleus and cytoplasm have been reported by Fukusawa, 1953, 1955, and 1957 and Kihara and Tsunewaki (1961). Working at Kansas Agriculture Experiment Station in U.S.A., Wilson and Ross (1962) established the existence of a stable and usable source of cytoplasmic male sterility in wheat. This was obtained from the interaction of the common bread wheat nucleus (variety Bison) with *Triticum timopheevi* cytoplasm. It has been usually observed that among the cytoplasms that interact with the Triticum nucleus, to produce cytoplasmic male sterility, *Triticum timopheevi* offers the best source and the possibility for the commercial seed production of the hybrid wheat. Unlike other sources of cytoplasm (*A. ovata* and *A. caudata*) no undesirable morphological characteristics have been observed to be associated with this source of cytoplasm.

**Genetics of fertility restoration**

Schmidt *et al* (1962) reported that the expression of fertility in the male sterile material obtained from Bison source would be effectively restored by crossing with a derivative of the cross *T. timopheevi* × *T. vulgare*. Livers (1964) reported that the fertility restoration mechanism in *T. timopheevi* cytoplasm was governed by two dominant complimentary genes $Rf_1$ and $Rf_2$. These genes were derived from the plant of a cross obtained from *T. timopheevi* × Marqius, a bread wheat variety. Both these two genes are necessary for the full restoration of fertility. However, when only one dominant gene is present, then fertility is only partially restored. The double recessive ($rf_1rf_1$ $rf_2rf_2$) condition is necessary for the expression of complete male sterility in *T. timopheevi* cytoplasm, *i.e.*, the sterile and maintainer both lines must possess the $rf_1rf_1$ $rf_2rf_2$ genetic constitutions.

**Problems in utilizing male sterility for hybrid seed production in wheat**

Some of the problems in the exploitation of heterosis in wheat have been the magnitude of seed set, pollen shedding of the male sterile parent as well as the pollen receiving behaviour of the seed parent under field conditions. Usually the *T. timopheevi* and *A. caudata* cytoplasm sterile lines usually produce 20-30 percent seed set. Most of the *A. ovata* steriles lines produce only 1-5 per cent seed set (Kherde *et al.*, 1964). However, about 9-63 per cent seed set has been reported by Livers (1964 b) with *T. timopheevi* cytoplasm. It was reported by Wilson and Ross (1962) that a range of 50-60 per cent seed set in seed production field may be expected with a ratio of one male pollen parent row to two seed parent rows under favourable conditions.

During the period of blooming, it has been commonly observed that in dense ears, angle of separation between lemma and palea is comparatively low as compared to the lax headed wheat (Wilson and Ross, 1962). It might be thus a limiting factor in the cross pollination. Some information about the role of the size of lodicules in the opening of spikelet may be needed to better understand the flowering process in wheat. Further research efforts would be needed for the understanding of the reasons for these problems and in overcoming them in the hybrid seed production before the production of hybrid wheat could be taken on a very large commercial scale.

**Steps involved in the production of hybrid wheat seed**

The availability of an established male sterile source with *T. timopheevi* cytoplasm, a cultivar of *T. timopheevi* strain with fertility restorer genes are two basic requirements for initiating a hybrid seed programme. The steps involved in the process of production are basically the development and maintenance of male sterile lines, incorporation of fertility restorer genes in male parent of the hybrid if not already present in it and production of the hybrid seed. A brief description of the steps is as follows :

i. **Development and maintenance of male sterile lines**

For the production of hybrid seed, the cytoplasmic male sterility is incorporated into the seed parent (line A) of the heterotic cross. This involves the crossing (Plate9.5) of an already available male sterile source and successively backcrossing the male sterile plants of the cross to seed parent until fully male sterile plants with the genotype of the seed parent are recovered. Usually 5-7 backcrosses are good enough. The original variety used as recurrent parent is then used as the maintainer parent of line A which is just like the seed parent except that it contains male sterile cytoplasm. Usually two lines of male sterile plant of line A with two lines of male fertile counterpart line B are grown in isolation and the seed is obtained only from seed parent. This is used as seed parent in the production of hybrid seed.

ii. **Incorporation of fertility restorer genes in the pollen parent (R line)**

The fertility restorer genes ($Rf_1$ and $Rf_2$) may be transferred to the pollen parent of the heterotic cross if already not present in it by the conventional backcrossing programme as described in the incorporation of male sterility. However, for effective detection of fertility restorer genes, the sterilization of the cytoplasm of the male parent on the availability of a linked gene with fertility restorer gene is a necessary requirement so that following each generation of backcrossing only the fertility restorer genes are carried forward in the backcrossed generations. The genetic constitution of A line, B line and R line with respect to their fertility restorer genetic background could be listed in the Table 9.4.

iii. **Production of hybrid seed**

For the production of hybrid wheat, usually two rows of A line are planted

side by side with one row of R line. Research findings indicate that in such an arrangement of rows usually 50-60 per cent seed set may be obtained under favourable conditions. Increasing the number of rows of A line results in poor seed setting. Watson and McWhirter (1968) taking advantage of seed size differences between the seeds of A and R line have suggested

TABLE 9.4

**Cytoplasmic and pollen fertility behaviour and genetic constitution of line A, B, R and hybrid utilized in the production of hybrid wheat**

| | *Parents* | *Genetic constitution with respect to fertility restorer genes* | *Cytoplasmic behaviour** | *Pollen fertility** |
|---|---|---|---|---|
| 1. | Seed parent (A line) | $rf_1rf_1rf_2rf_2$ | S | S |
| 2. | Maintainer line (B line) | $rf_1rf_1rf_2rf_2$ | F | F |
| 3. | Fertility restorer line (R line) | | | |
| | a. Obtained from *timopheevi* source | $Rf_1Rf_1Rf_2Rf_2$ | S | F |
| | b. Obtained from *aestivum* source | $Rf_1Rf_1Rf_2Rf_2$ | F | F |
| 4. | Hybrid | $Rf_1rf_1Rf_2rf_2$ | S | F |

* Fertile (F) and Sterile (S)

that the equal quantities of the seeds of both these lines of a heterotic cross could be mixed together and this bulk is planted as the commercial crop. The seed harvested from this crop, could be sieved out and the hybrid seed could be separated from the bulk. Recently use of colour marker genes has also been suggested to be incorporated in the A and R lines of the hybrid in the place of differences in the seed size of the seed and pollen parent and the bulk seed is grown. With the bulk seed with two distinct colours, representing one of the hybrid and other of R line could be separated from the bulk with the help of colour sensitive separators and the seed could be produced commercially on a large scale.

**Genetic male sterility and hybrid seed production**

A summarized information regarding the genetic male sterility in crop

plants has been given in Table 9.5.

TABLE 9.5

**Genetic male sterility in crop plants**

| *Sl. No.* | *Crops* | *Marker gene if any* | *Authors* |
|---|---|---|---|
| 1. | Barley | — | Suneson (1945), (1951) Weibe (1960) |
| 2. | Carrot | — | Hansche and Gabelman (1963) |
| 3. | Cotton | — | Justus and Leinweber (1960), Kohel and Richmond (1962), Allison & Fisher (1964) |
| 4. | Lettuce | Narrow, sharply cut leaves | Lindqvist (1960), Ryder (1963) |
| 5. | Maize | — | Beadle (1932) |
| 6. | Sorghum | — | Stephens (1937) |
| 7. | Sunflower | — | Putt and Heiser (1963) |
| 8. | Watermelon | glabrous leaves | Watts (1962) |
| 9. | Wheat | — | Suneson (1962), Athwal *et al.* (1967), Athwal & Borlaug (1967) |

This type of male sterility could be utilized in hybrid seed production pragrammes. However, unlike the cytoplasmic one, it does not eliminate all the manual labour. Since most of the male sterility genes are recessive (ms ms); in terms of Mendelian genetics, they are maintained by crossing the ms ms with Ms ms. They then usually segregate into the ratio of 1:1 for Ms ms and ms ms. A strain thus, will never be or remain completely sterile. The rouging of the fertile plants would, therefore, be universally necessary before the plants start shedding their pollen grains. This type of male sterility if it is used as the female parent in the hybrid seed production programme, then about 50% of the male fertile plants must be rouged out prior to their flowering and or pollen shedding. The tedium of removing such a large number of plants where possible could be eased with the uses of (a) some closely linked marker genes; or (b) by mass screening technique for the quick elimination of male fertile plants in terms of some male gametocidal sprays etc. The possible marker genes linked with male sterility have been reported in watermelon (Watts 1962), Lettuce (Lindqvist 1960), and rapeseed (Tyagi *et. al.* 1978).

Weibe (1960) theoretically suggested a mass screening technique in barley with the use of a possible marker gene for resistance of DDT spray linked with male sterility factor. But such a linkage in barley is not yet available.

Genetic male sterility, however, could be effectively used as an aid to the cytoplasmic male sterility where the commercial product is not the seed but

the vegetative part under the situations where a strong fertility restorer gene system is not readily available. A case in this respect has been given by Owen (1952, 1954) in sugarbeet.

In sugarbeet, usually double crosses are produced and used as the commercial hybrids. The first single cross namely (A×B) could be produced by the use of cytoplasmic male sterility and the other namely C×D by the use of genetic male sterility. As only a small proportion of male plants are needed in the double cross sugarbeet seed production, small quantities of male single cross seed would be needed. This however, would eliminate the need of developing a very strong fertility restorer in D inbred of the C×D single cross. At the same time, it would not affect the production schedule in this crop since the commercial harvestable products is not the seed but the root of the plant.

**Pistillateness and the hybrid seed production**

In some of the normally monoecious plants like castor in the presence of certain modifier genes alters the expression of male flowers in the inflorescence and produces completely pistillate plants. Such a situation could possibly be utilized for using these plants as the seed parent in the hybrid seed production. Shifriss (1960) reported such a situation in castor. He suggested that lines with dominant factor for pistillateness alongwith a recessive gene for few male flowers whose expression is entirely environment controlled (*i.e.*, it could be maintained by self or sib pollination in an environment where it expresses), could be used as the seed parent in other environment where the recessive gene for male flowers is supressed. He also selected a homozygous recessive female (ff) genotype which would produce only occasional male flowers. This line was entirely female in some environment but not in the other. Such a behaviour of the line could be advantageous *i.e.*, the one for the maintenance of the line and the other for its use as a seed parent in the hybrid programme. In India the castor hybrid seed production programme is currently under way and a number of commercial castor hybrids are now available. These hybrids are produced by the use of practically pistillate lines especially in the state of Gujarat. The buds of a few flowers which occasionally appear on the plants of the seed parent are elliminated by the manual labour. The seed set on the seed parent is harvested, processed and delivered for the commercial cultivation of this crop.

**Artificially induced male sterility and the hybrid seed production programme**

The artificially induced male sterility if effective, efficient and economical may be an aid to the hybrid seed production programme, especially where such a natural system is readily not available. The various methods for inducing male sterility in crop plants have been the use of gametocidal sprays, use of radiation and even aneuoploidy. A brief discussion of some of these have been given as follows :

(a) *Use of gametocidal sprays* : In crop plants, a number of selective male gametocides which selectively kill the male gametes without any way injuring or affecting the female ones, have been tried with varying degree of success. Some of these have been FW 450 (Sodium-2, 3, dichloroisobutyrate), phosphone (Tributyl-2, 4, dichlorobenzyl ammonium chloride), potassium gibberllate, ulepan (a Dichloropropionic acid derivative) and even colchicine.

In cotton, (*G. hirsutum*) variety B-49, Bouharmont and Pochet (1965) at Bambesa Station in Congo used FW 450 to induce male sterility. They observed and reported that the 1.5% concentration of the solution was effective in bringing about complete male sterility for a duration of 35 days approximately after 20 days after the spraying. But it was simultaneously accompanied with a 30 to 40% decrease in female fertility when these plants were fertilized with pollen from another cotton variety. Lower concentration of FW 450 though comparatively less effective, however, resulted in greater number of hybrid plants because of the high female fertility. The hybrid seed produced by this method showed atleast 93% normal germination. The above workers recommended that spraying of these chemicals is easy and could be effectively utilized to eliminate the tedium of hand emasculation in the hybrid seed production of this crop species.

Spraying of FW 450 on cucumber caused some reduction in size and pollen viability and brought about some functional male sterility (Mihov and Genchev 1966). In raddish, this chemical was tried in the concentration of 0.5%, 1.0% and 1.5%. Spraying with 1% solution resulted in an overall hybrid seed production of 33.3-50 % on the seed parent. However, in a crop like raddish this was not thought to be adequate to warrent its commercial usage in a viable hybrid programme.

Cohan and Wiegle (1966) used gametocidal sprays of phosphone to induce male sterility in an onion inbred Iowa-90. The sprays were applied 1½ weeks prior to anthesis in the concentration of 750 and 1000 ppm. They suggested that for obtaining the complete male sterility repeated sprayings would be needed. James and Lund (1965) applied potassium gibberlate solution (5000 ppm) at anther initiation in barley and obtained encouraging results. They reported that practically similar results could also be obtained by repeated sprayings of this chemical with 500 ppm alongwith 20 ppm of L—naphthalene acetic acid and 100 ppm 2, 3, 5-triindobenzoic acid during the period of 10 days prior to heading time. This was, however, more effective in the winter barley than in the spring barley varieties.

Ter-Avanesgan (1964) reported 90-95% male sterility in cotton by repeated spraying with 1% acquous solution of ulepan (a derivative of dry chloropropionic acid) in the mid season types 108F, 138F and S 450. In sorghum Erichsen and Ross (1963) reported rather an unusual case in which colchicine treatment caused mutation of normal to sterile cytoplasm of a milo type. This is perhaps the first reported case of colchicine treatment causing muta-

tion to sterile cytoplasm in this crop. Almost similarly Siddiq (1967) has reported two totally sterile dwarf plants among the treated seedling of the sorghum hybrid ms CK 60 × IS 1054 by colchicine treatment and attributed this phenomenon to mutation.

(b) *Mutagen and aneuploidy induced male sterlity* : Moutschen-Dahmer (1965) reported sterility induced by EMS (Ethyl-methane Sulphonate) and x-rays in barley. He observed that at an equivalent survival levels, EMS caused a much higher degree of sterility than the X-rays. Kazakova (1965) utilized X-ray to obtain male sterile forms in onion and used them for the hybrid seed production in USSR. She reported that X-rays doses of about 5000r proved lethal to the bulbs and were accompanied with various growth abnormalities.

Aneuploidy has recently been viewed as a potential aid in inducing male sterility in some of the major economic crop species for the commercial hybrid seed production. It has usually been observed that either substitution or even loss of a particular chromosome from one variety to other upsets the genic balance and causes sterility. In Oats, Lafaver and Patterson (1964) suggested the use of male sterile aneuploid as seed parent to produce the hybrid seeds. However in self pollinated crops there are many other problems in the way of the exploitation of heterosis and the hybrids in such crop are still a distant reality.

To sum up the male sterility both the natural and the artificially induced types have their own merits and demerits. There is however, no substitute for a natural source for hybrid seed production. Though rare, it is cheap, easy and convenient to handle. The artificially induced male sterility eliminates the need of a fertility restorer mechanism but is usually not completely efficient, costly and sometimes affect this seed fertility and also the seed germination. However, it is usually viewed that where one fail other may take over. Wherever possible it may supplement, combine and be simultaneously assessed and utilized for its economic feasibility in a viable hybrid seed programme.

**Self incompatibility and hybrid seed production**

Self incompatibility refers to the inability of a plant to set seed when self pollinated with normal functional pollen and ovules even though it can set seed when cross pollinated. The major reason for this is the slower growth and failure of the pollen tube to grow down and penetrate the style as compared to the pollens from genetically dissimilar plants. This is because of the presence of certain growth inhibitor substances present in the styler tissue which arrest the growth of the pollen of the same genetic constitution.

Self incompatibility basically is an outbreeding mechanism. It promotes hybridity and is of common occurrence in plant kingdom. E.M. East estimated about 35 years ago that it occurs in more than 300 species among 20

families of the flowering plants. It is of the common occurrence in crop species belonging to the family composite (sunflower, cosmos) cruciferae (rapeseed, raddish, cabbage, cauliflower etc.), graminae (forage grasses), leguminosae (forage legumes) and solanaceae (petunia, potato, tobacco, certain tomato relatives etc.) (Table 9.6).

TABLE 9.6

**Self incompatibility mechanism in crop plants**

| | *Crop species* | *Families* | *Type* | *Authors* |
|---|---|---|---|---|
| 1. | Alfalfa (Lucerne) | Leguminosae | - | Whitehead and Davis (1954) |
| 2. | Alsike | Leguminosae | - | Williams (1951) |
| 3. | Broccoli | Cruciferae | - | Odland (1962) |
| 4. | Bromus | Graminae | - | Admas (1954) |
| 5. | Festuca | Graminae | - | Lundqvist (1955) |
| 6. | Kale | Cruciferae | Sporophytic | Thompson (1957) |
| 7. | Rapeseed | Cruciferae | Sporophytic | Bateson (1955) |
| 8. | Red clover | Leguminosae | - | Leffel (1963) |
| 9. | Rye | Graminae | Gametophytic | Lindqvist (1956) |
| 10. | Sunflower | Compositae | Sporophytic | Habura (1957) |
| 11. | Tomato | Solanaceae | - | Martin (1964) |

## Genetics of self-incompatibility

Self-incompatibility mechanism is a genetically controlled phenomenon. It is governed by a series of S alleles. If the gene present in the pollen is identical with the allele present in the styler tissue, then there is the failure of fertilization and self incompatibility consequently occurs. In various crop families usually two types of self incompatibility mechanisms are found. These are (i) gametophytic type and (ii) sporophytic type. It is noticed that in each crop families usually one type occurs.

In gametophytic type of self incompatibility, a pollen grain which carries a given self incompatibility gene (say $S_1$) cannot function on a pistil which possess the same allele. In this type the control of self incompatibility is gametophytic. Usually, S alleles are independent in their action and there is no dominance relationship between them. With the presence of 4 S alleles ($S_1S_1$, $S_2S_2$, $S_3S_3$ and $S_4S_4$), the gametophytic type gives rise to three types of pollinations (i) fully incompatible ($S_1S_2 \times S_1S_2$) in which both alleles are common, (ii) half the pollen is compatible ($S_1S_2 \times S_1S_3$) in which one allele is different and (iii) all the pollen is compatible ($S_1S_2 \times S_3S_4$) in which both

alleles differ (Fig. 9.2) (Lewis, 1954). This type of self incompatibility is usually present in crops like tobacco, lucerne, rye etc.

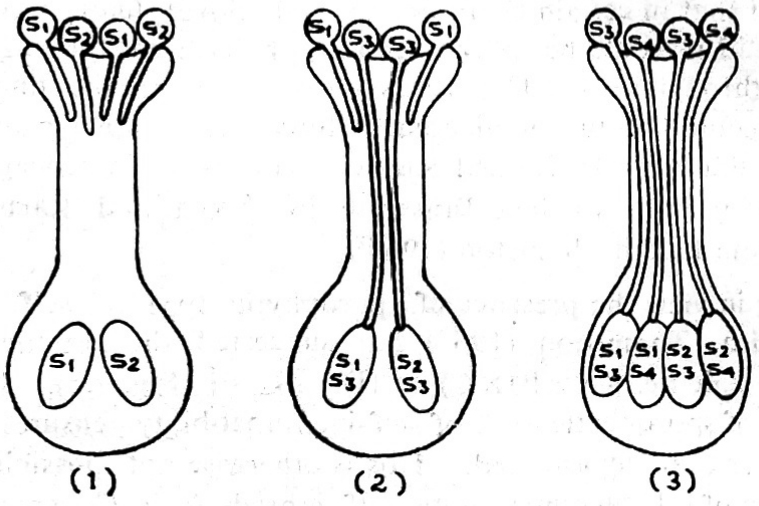



*Fig. 9.2* : Incompatibility patterns obtained with the gametophytic system of self-incompatibility.
(1) Fully compatible both alleles are common
(2) Half pollen compatible. One allele is different and
(3) All pollen compatible both alleles are different.
After Lewis (1954) and Poehlman and Borthakur (1968).

In sporophytic type of self incompaitbility, unlike the gametophytic type, the genetic control and pollen reaction of S allele is controlled by sporophytic mechanism. The alleles often show dominance relationship. There are reciprocal differences between the crosses. Homozygotes are usually formed. Consequently, all the pollen obtained from a heterozygous plant react similarly irrespective of whether the plant that bears them is heterozygous or homozygous for self incompatibility alleles. This type of self-incompatibility is usually present in crops like rapeseed, cabbage, kale, sunflower etc.

**Hybrid seed production**

The self incompatibility mechanism whether gametophytic or sporophytic present rather a contradictory feature to the plant breeder in the development of commercial hybrids. On one hand it frustrates efforts to produce homozygous inbred lines. The inbred lines produced in self incompatible crops are usually very weak and produce very less seed. However, on the other hand it provides a natural mechanism to hybridize two lines without emasculation and without resorting to genetic or cytoplasmic male sterility. It could, therefore, be utilized for making controlled pollination in a hybrid seed production programme (Duvick, 1966).

For the production of commercial hybrid in red clover, Leffel (1963) suggested the production of double cross hybrids $(S_1S_1 \times S_2S_2) \times (S_3S_3 \times S_4S_4)$ utilizing self incompatibility alleles $S_1S_1$, $S_2S_2$, $S_3S_3$ and $S_4S_4$. In this plan

each inbred must be self incompatible enough to produce all hybrid seed by outcrossing with another line. Likewise, each single cross, thus produced should be self incompatible but cross compatible with other single cross. He suggested that in certain environments red clover lines become pseudo-compatible and it could be advantageously be utilized for maintaining the inbred lines through seed. The process of maintaining the lines by vegetative method could be thus eliminated. More or less similar plants have been suggested in alfalfa by Tysdal and Kiesselbach (1944), in cabbage by Odland and Noll (1950), in oil yielding Brassicae by Sikka and Rajan (1957) and in marrow stem kale by Johnston (1964).

Keeping in view the presence of sporophytic type of self incompatibility in Brassica, Thompson (1964) has suggested the production of triple cross hybrid kale *i.e.* $[(A \times B) \times C] \times [(D \times E) \times F]$ (Fig. 9.3). In this plan the presence of sporophytic type of self-incompatibility ensures the production of 100 per cent hybrid seed. This is otherwise not possible with gametophytic type of self incompatibility. If inbreds A, B, C were of genotypes

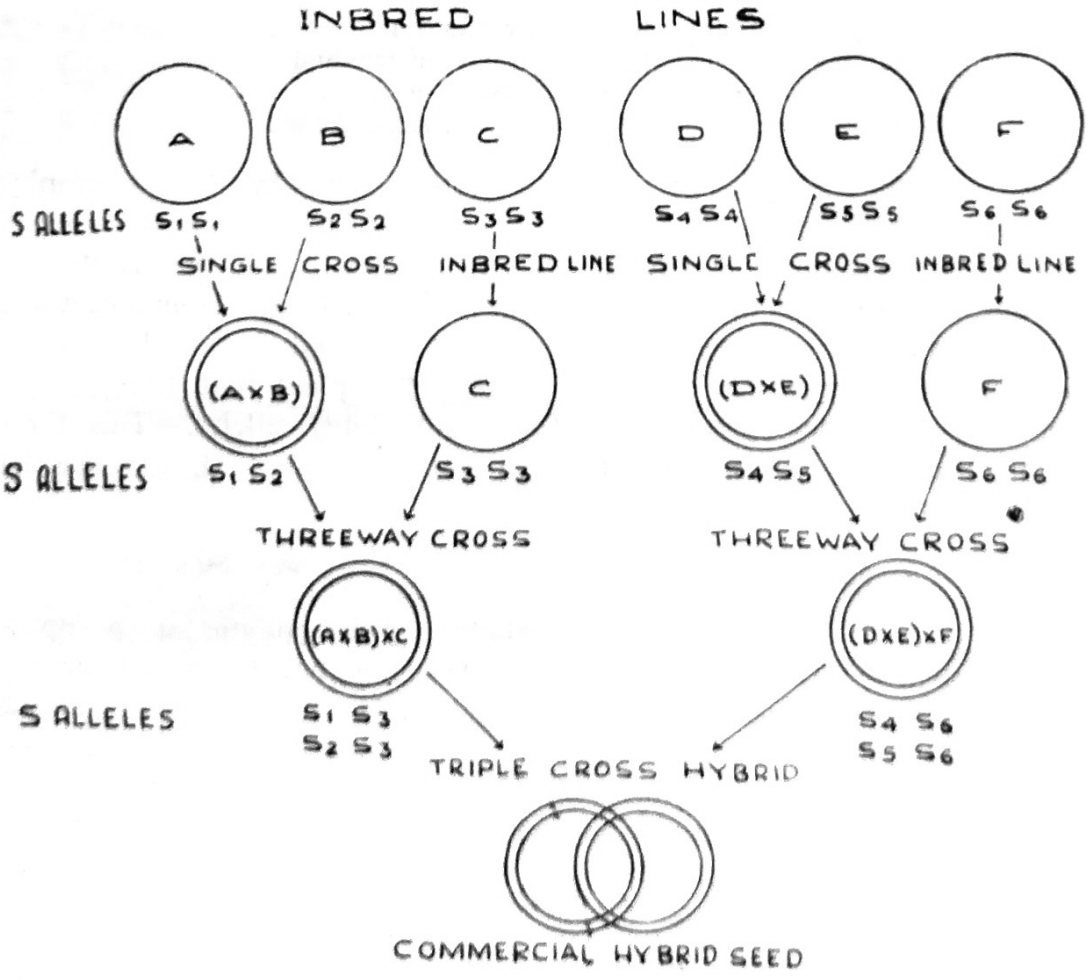



*Fig. 9.3* : Production of triple cross hybrid kale utilizing sporophytic type of self-incompatibility (After Thompson 1964).

$S_1S_1$, $S_2S_2$ and $S_3S_3$, the single cross $A \times B$ would then be $S_1S_2$ and three way cross $(A \times B) \times C$ would be having $S_1S_3$ and $S_2S_3$ genotype. With sporophytic type, if $S_3$ were dominant in pollen and style over $S_1$ and $S_2$ genotypes, the genotype $S_1S_3$ and $S_2S_3$ would be self incompatible because of dominance of $S_3$ over $S_1$ and $S_2$ alleles. This could thus safely be used as seed or pollen parent for seed production. With gametophytic system of self incompatibility, it would be rather impossible to produce 100 per cent hybrid seed because some plants in each three way cross with $S_1S_3$ and $S_2S_3$ genotypes would be cross-compatible. This is because of the independent behaviours of $S_1$, $S_2$ and $S_3$ alleles and absence of dominance relationship between them.

The triple cross has been reported to be advantageous. It reduces the extent of bud pollination to maintain inbred lines, utilizes vigourous plants for seed production, gives high amount of seed, provides superior winter survival, and provides broad genetic base for the hybrids. Thus the hybrid seed is produced at rather a lower cost as compared to the single crosses. The limitations of this method, however, has been reported to be that with 6 inbred lines involve in the production, the performance of the hybrids based on the standard method of the double cross prediction as it is usually done in the case of maize hybrid would, however, be difficult here and could not be carried over. This is because of fact that if the C and F lines with dominant gene for self incompatibility be interfering with performance of $(A \times B) \times (D \times E)$, it is less likely that any of the two lines A or B could replace C or D or E could replace F. However, with the obvious advantages of the triple cross, this may not necessarily be a major limitation and could atleast be partially eliminated by wider choice of productive and high combining inbred lines in the production of triple cross hybrid.

10

# Breeding Synthetic and Composite Varieties : A Way for the Partial Utilization of Heterosis

**The need**

The commercial production of the hybrids provides an opportunity for the complete exploitation of heterosis. This is because the matings of the seed and the pollen parents are done under isolation and artificially controlled conditions and practically 100% hybrid seed is delivered to the farmers for commercial production programmes. Wheresoever, this is possible, it is perhaps the ideal situation, however, sometimes the control of the parentage to produce the hybrid seed is not possible because of a number obvious reasons. Some such reasons for the non-exploitation of full heterosis in crop plants are the biological or economical limitations. The biological limitations may be the unamenability of the flower structure and its biology. Extremely small size of the flowers sometimes make the controlled crossing very difficult. The non-availability of a useful male sterility system as one sees in some of the grasses, oil yielding Brassicas and several of the forage legumes make the commercial production of the hybrid seed very difficult in such crops. Lack of organized skillful seed production may be a serious bottleneck also. An adequate quantity of fresh hybrid seed is needed every year. If the distribution system is combersome, the hybrid may not usually reach the farmer in time for planting and may become a deterrent to the production programmes. Also if the hybrid seed produced for a crop is grown in small acreage of land, scattered over several small areas with poor rainfall and inadequate transportation facilities, may not tempt or economically sustain the hybrid seed industry. The cost of hybrid seed if it is much more than the usual planting material (4 to 10 times), it may discourage their uses by small farmers with small acreage and poor means. In developing countries like India some of the farmers knowingly or unknowingly may not be able to meet the exact rate of high doses of fertilizer, pesticides and other inputs which hybrid varieties usually demand. Under such situations, the breeding of synthetic and composite varieties may be useful. These would help to obtain considerably good level of production with the farmer's own seeds.

**Synthetic and composite varieties**

The synthetic varieties are technically the advanced generation, open pollinated seed mixture of a number of crosses obtained from a number of inbred lines tested for their combining ability and which has been grown in isolation. Hayes (1963) defined the synthetic varieties as the open pollinated varieties obtained from multiple hybrids. Recently the term composite variety* is being frequently used. In principle, this term could be classified broadly under the synthetic variety, however, now it is generally used to cannote the advance generation seed mixtures obtained from the intervarietal or multiple varietal hybrid crosses or their blends. The composites are usually developed from the varieties or other heterozygous base populations or germ plasm which have ordinarily not been subjected to inbreeding or have not been elaborately tested for their combining ability, Singh (1965). Usually they include various breeding materials like varieties synthetics, double crosses, races etc. based on their yielding performance, maturity time, grain characteristics, disease resistance and other such known characteristics. A general comparison of this two types of varieties has been given in Table 10.1.

TABLE 10.1

**Major distinguishing characteristics of the synthetic and composite varieties**

| | *Characteristics* | *Synthetic varieties* | *Composite varieties* |
|---|---|---|---|
| 1. | Base populations | Inbred lines | Varieties or other heterozygous sources |
| 2. | Testing for general combining ability | Tested | Usually untested |
| 3. | Number of constituent inbreds/varieties | 4 - 20 | 2 - several hundreds |

**Usefulness of synthetic and composite varieties**

Synthetic and composite varieties though theoretically are less yielding than the hybrids but have been often observed to give better performance over the local open pollinated varieties (Wellhausen 1952). Synthetic varieties have often observed to be superior in fibre and yarn strength and yield in cotton (Duncan, Pate and Turner 1963).

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* *The word composite variety has been used by Harlan and Martini (1929) and Suneson (1949) (1956). What they meant by this term was the equal quantity mixture or blend or the seed mixtures of a number of variety in a crop like barley. The crosses between a number of varieties which were mixed equally from each crops was called the Composite Cross.*

Sprague and Jenkins (1943) and Dhawan (1965) reported that synthetic and composite varieties might have value as the reservoir of desirable germplasm. They noted that these varieties might be of considerable values on the fringes of maize growing areas where the cost of hybrid seed is relatively high than the value of the expected crop. The greater variability of the synthetic varieties as compared with the double cross hybrids might permit more flexibility to meet the changeable growing conditions of the marginal areas. These varieties have a place where commercial acreage is too small to support a hybrid maize seed industry.

## (A) BREEDING SYNTHETIC VARIETIES

### (i) *Genetic Basis of Breeding Synthetic Varieties*

It is now an established fact that the hybrid breeding programme capitalises predominantly on the non-additive gene system. According to the genetic theory, non-additive effects (overdominance and epistasis) are to a great extent dissipated in the $F_2$ and the $F_2$ performance is largely the result of additive effects. Under the condition of random mating, it would largely remain stable.

The breeding for synthetic varieties capitalises predominantly on the additive gene system. It has been usually observed that in the consequent self generations of both hybrids and the synthetics the quantum of inbreeding depression is much more in the hybrids than in the synthetics. This indicates to the fact that hybrids have more of non additive gene effects than the synthetic variety. Usually if the non additive gene system is involved, the breakdown due to inbreeding in $F_2$ would cause considerable depression but if it is predominantly additive system it would be comparatively less. After three or four generations, it would stabilize and there would be no appreciable change in the performance.

Sewall Wright in 1922 suggested the following formula for estimation of the performance of the $F_2$ generation

$$\hat{F}_2 = F_1 - \frac{(\bar{F}_1 - \bar{P})}{N}$$

where $\hat{F}_2$ is the estimated performance of $F_2$ generation, $\bar{F}_1$ is the average performance of all the single crosses among the parental lines involved, $\bar{P}$ is the average performance of the parental lines and N is the number of parental lines. As given in the formula, the component $(\bar{F}_1 - \bar{P})/N$ is the amount equal to 1/n of the difference between the $F_1$ and the average of the parental lines. In other words, it is the indicator of the amount of non-additive gene action involved. In a population where additive gene action is complete, it would turn towards zero and there will be no inbreeding depression at all. For example in a case of hybrid giving 45 quintal/ha. with the inbreds each giving 40 and 50 q/ha. under the complete additivite gene

system, the predicted performance of the $F_2$ would be

$$F_2 = 45-\frac{[45-(40+50)]}{2}$$

$$= 45-(0) = 45 \textit{ i.e.}, \text{ no decline at all.}$$

$$= 45$$

But usually this is seldom the case. Some non additive gene action is always inherent alongwith the additive gene action in the expression of a quantitative characteres. The actual difference between the estimated and the observed performance will indicate the loss of non additive gene action. For example in the case of a hybrid giving 50 q/ha. obtained from the inbreds giving 30 and 36 q/ha. the estimated $F_2$ performance would be

$$F_2=50-\frac{(50-(30+36)/2}{2}$$

$$=50-8.5=41.5 \text{ q/ha.}$$

In the next generation in this hybrid there would be an expected loss of 8.5 q/ha. This shows that theoretically, the synthetic varieties will yield less than the corresponding hybrids.

The experimental evidences on the type of gene action involved in the breeding of the synthetic varieties have been obtained from the study of the genetics of quantitative characters in maize. Lonnquist and Rumbaugh (1958) experimentally analysed the effect of selecting maize lines for their general and specific combing ability and their successive usefulness in a synthetic breeding programme. They synthesised synthetic varieties based on the lines obtained from the parental sources selected on the basis of general combing ability as also the specific combining ability. From the parental source of Krug Synthetic I, a new synthetic K II was constituted based on the specific combing ability of $S_0$ lines. Most $S_0$ lines were crossed to a common tester. The results obtained showed that the K I and K II did not differ much. This was attributed to the selection of $S_0$ lines predominantly on non additive gene action but not the additive gene action upon which the improved yield of a synthetic variety usually depends. To prove this point, they also selected lines based on their general combining ability and reconstituted another synthetic varieties known as K IIA. The yield of K IIA was observed to be superior to both K I and K II. This showed that the selection of lines having higher general combining ability or pre dominantly additive gene action is more advantageous for higher expression of yield in a synthetic breeding programme than to the lines selected on their specific combining ability or predominantly non- additive gene action.

(ii) *Steps involved in the development of synthetic varieties*

Synthetic varieties may be developed either straight from $S_0$ or $S_1$ plants as

short term plan or may be developed from the well established tested inbred lines. A brief description of the above two methods have been given as follows :

(a) **From $S_0$ or $S_1$ Plants :**

The use of $S_0$ or $S_1$ plants in the development of synthetic variety has number of advantages. The testing for combining ability can begin with the selected open pollinated plants which may simultaneously be selfed and crossed with. After the first generation of selfing the plants or lines may be immediately utilized in the development of synthetic varieties. Usually, it has been observed that the $S_0$ or $S_1$ plants are vigorous than the established lines and may have a definite advantage in synthetics where genetic uniformity is not of prime consideration. The data obtained in the Mexican Maize Breeding Programme indicate a high degree of relationship between the performance of $S_1$ and the average performace of lines obtained from each subsequent generation of inbreeding. A correlation coefficient of +0.69 was obtained between the top cross yield of $S_1$ lines and the average top cross yield of $S_2$ lines derived from each of 138 pairs of value. As a result of elaborate tests, it was concluded that tests for combining ability in the $S_0$ or $S_1$ generation would serve to separate the families that are good combiners from the family that are poor combiners in the early stages of the inbreeding programme (Wellhausen 1952).

The various steps involved in the development of synthetic varieties from $S_0$ or $S_1$ lines constitutes of (1) isolation of one generation selfed lines (2) testing these lines in top cross trials for yield and other characteristics (3) intercrossing the better line *inter se* to produce synthetic variety and (4) the repetition of the above cycle at intervals after a generation or two of open pollination (Jenkins 1940). This method is based on the fact that the combining ability of the lines is fixed in the early generation of inbreeding and they could be identified by the top cross performance. There would, as a matter of fact, be gained little by additional selfing and selection when the objective is to develop basically a heterozygous synthetic variety. A number of synthetic variety have been developed by utilizing $S_0$ plants in maize (Lonnquist and McGill 1956) and have been found to be better in yield performance.

**Use of polycross in the development of synthetic varieties from $S_0$ plants**

In some of the small flowered cross pollinated crops, where elaborate hand crossing is difficult to test the general combining ability of $S_0$ plants through top crossing, the use of polycross procedure may be followed mainly because of its ease and economy of effort.

Polycrossed progenies are the progenies of a line obtained as a conseqence of out crossing with a selected group of lines growing in the same nursery. The lines are grown at random with a minimum of 10 replications in a poly cross nursery, free outcrossing is allowed and the seed obtained

from each line is bulked and grown next year in a replicated trial to evaluate the relative performance of the lines. This has been found to be reliable method for estimating general combining ability in various crop species. A comparative performance of various lines under both poly cross and top cross tests showed that if the best 20% of the lines $S_0$ plants or clones are to be selected, the same lines would be choosen on the basis of anyone of these two tests (Tysdal 1948) (Tysdal and Crandall 1948, Murphy 1942, Maini, Singh and Labana 1960 and Maini and Ghai 1964). Because of its simplicity, poly cross is a popular method of screening inbred lines or $S_0$ plants for their general combining ability and has been adequately used in the breeding of synthetic varieties (Tysdal, Kiesselbach and Westover 1942, Tysdal and Crandall 1948, Maini et al. 1960, Rai and Mallik 1970).

The basic steps in the development of multi-line synthetic variety as outlined by Poehlman and Borthakur (1969) has been given as follows and been diagrammatically presented in Fig. 10.1.

Step 1. Assemble several thousand genetically variable plants. Inbreed one or more generations a number of superior looking plants and visually select 200 - 400 of them.

Step 2. Screen the plant for resistance to diseases, insects and lodging and for various other agronomic characters, and select 25-50 for further testing.

Step 3. Grow 25 - 50 lines selected in the Step 2 in a poly cross nursery. Allow open pollination and harvest each plant separately and then bulk the individual poly cross progenies.

Step 4. Evaluate the poly crossed progenies in yield trial and select 4 - 10 superior combining lines/plants.

Step 5. Constitute a synthetic with these 4 - 10 lines suggested from Step 4 by bulking the equal quantity seed from each. Open pollinate and evaluate with the standard check or the local variety.

Step 6. Repeat the cycle or start a recurrent selection programmes if the requisite amount of improvement has not been obtained.

A number of synthetic varieties in forage crops (timothy, orchardgrass and lucerne) have been developed following this method at the Welsh Plant Breeding Station Aberystwyth and are now in commercial use in Great Britain (Jenkins 1949).

### (b) Development of synthetic varieties from established inbred lines

The various steps involved in the development of synthetic varieties from the established inbred lines constitutes of (1) testing them for general combining ability by top cross with a suitable tester parent; (2) inter-crossing a number of best combining lines (4 - 20) in all possible combinations growing and

evaluating their performance; (3) predicting the $F_2$ performance of various combinations; (4) evaluating the performance of experimental synthetics alongwith the checks and the local variety over years and locations and finally picking the best ones for release in the various agro-climatic tracts. A detail account of these steps is as follows :

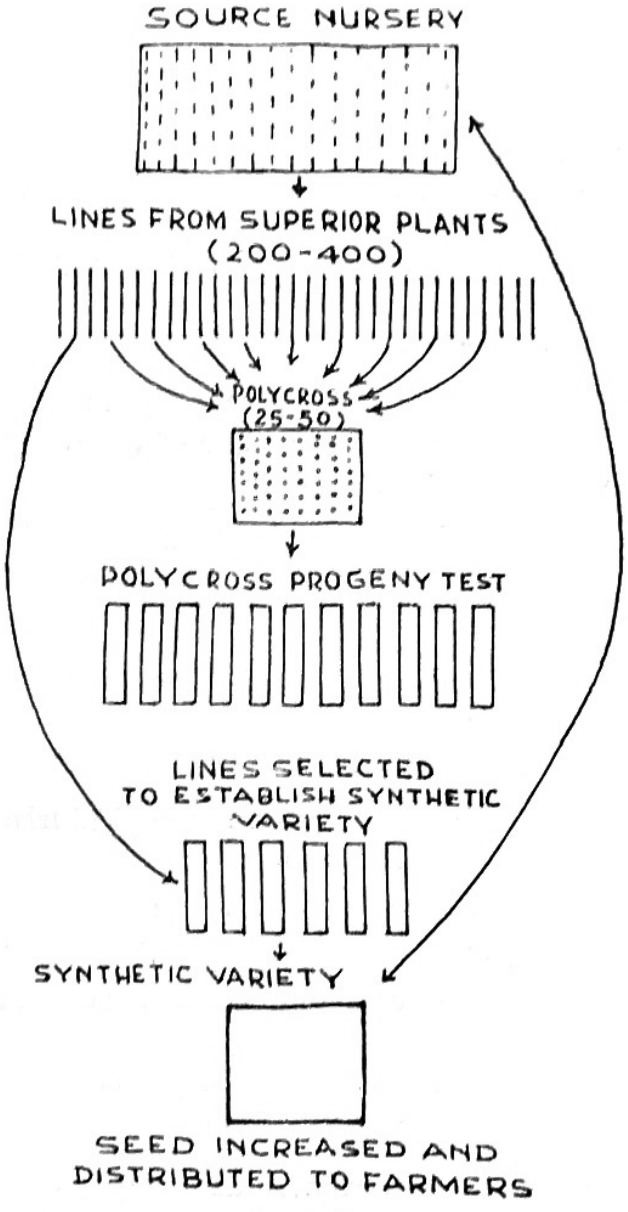


*Fig. 10.1* : Basic steps involved in the polycross testing for developing a synthetic variety (After Poehlman and Borthakur 1968).

### (i) Testing inbred lines for general combining ability

In breeding for synthetic varieties, usually the testing of inbred lines for general combining ability is done in the $S_4$ (fourth self generation) or later generations of selfing and selection. The various methods of testing inbred lines is the use of top crosses, poly crosses or the diallele cross. Top cross

is usually used for the large scale screening of the inbred lines in maize. The necessary consideration in the use of top crossing, however, is the selection of an appropriate tester.

**Type of tester parent for breeding synthetic varieties**

The screening of well nicking genotypes (inbreds) through top crossing from genetically variable lines to combine into a synthetic variety involves the right choice of a suitable tester parent. The best tester is usually the one which maximises the expected mean yield of a synthetic variety produced by random mating of selected genotypes. Usually the criteria for selection of a suitable tester parent has been that it should be genetically heterozygous *i.e*, the type of tester usually advocated for measuring general combining ability. The parental populations as compared to other open pollinated varieties with contrasting gene frequencies are safer to use as tester parent (Lonnquist and Lindsay 1964).

(ii) **Making single crosses**

The next step after testing the inbred lines for general combining ability is making all possible single crosses between a number of selected lines as is usually done in the breeding of hybrid maize. As a matter of fact, the breeding of hybrid maize and that of synthetic varieties may even run parallel. The single cross data obtained from the double cross breeding programme may equally well be utilized for this purpose. Perhaps, one of the most ideal and logical procedure would be the development of the double cross hybrids which would also make good synthetics in advanced generations.

After making the single crosses, they are grown into a replicated yield trial alongwith the parental inbred lines. The data is obtained on the average performance of the single crosses and the parental inbred lines. From this data, the predicted yield of the $F_2$ generation of the synthetic varieties with the different number of inbred lines is obtained using the formula given by Wright (1922).

(iii) **Predicting the relative $F_2$ performance of synthetic varieties**

The prediction of the performance of various combinations of inbred lines is done to establish the number of componental lines that may be marshalled into the making of a particular synthetic variety. This is usually done by arranging the lines in the descending order according to their mean combining ability performance. Then starting from the first two or four, the predicted yield of the synthetics are obtained. For example, assuming the mean performance of four inbred lines A, B, C and D and their all possible single crosses (A×B), (A×C), (A×D), (B×C), (B×D), and (C×D) is respectively 67, 65, 62, 60 q/ha. for the inbreds and 85, 88, 90, 75, 76 and 80 respectively for the hybrids, the predicted yield of synthetics with four inbred lines according to Wright's formula, would be

$$\hat{F}_2 = F_1 - \frac{(\bar{F}_1 - \bar{P})}{N}$$

$$= \frac{85+88+90+75+76+80}{6} - \frac{\frac{85+88+90+75+76+80}{6} - }{4}$$

$$\frac{67+65+62+60}{4} = \frac{85.6-(85-6-63.5)}{4} = 85.6-5.5=80.1$$

Similarly, the predicted performance of synthetics with the differing combinations of lines is theoretically obtained and compared. This formula has been tested by a number of workers (Neal 1935, Kisselbach 1933, Wellhausen and Roberts 1949) to predict the performance of synthetic varieties. The expected and the observed yield of $F_2$ populations as computed by Neal (1935) has been given in Table 10.3.

TABLE 10.3

**Actual and predicted yield of maize obtained in $F_2$ populations (After Neal 1935)***

| *Sl. No.* | *Type of crosses* | *No. of crosses* | *Average yield* $F_1$ | $P$ | *Difference* $F_1$ - $P$ | *Yield of $F_2$ (q/ha.)* | |
|---|---|---|---|---|---|---|---|
| | | | | | | *Expected* | *Actual* |
| 1. | Single crosses | 10 | 42.1 | 15.9 | 26.2 | 29.0 | 29.6 |
| 2. | Three way crosses | 4 | 43.0 | 15.9 | 27.1 | 34.0 | 33.0 |
| 3. | Double crosses | 10 | 43.0 | 16.7 | 26.2 | 36.4 | 36.2 |

* *Values adapted in q/ha. from bu/acre.*

As is apparent in Table 10.3, the prediction of $F_2$ performance based on the Wright's formula closely fits with the actual performance. It also shows that (a) the actual and predicted yields of various hybrids are not greatly different and (b) that with the increasing number of lines from 2 to 4 also increases the expected $F_2$ yields and it closely fit with actual performance.

The procedure to exactly determine the optimum number of constituent inbred lines which may go in the maximisation of the performance of a synthetic variety were worked out in detail by Kinman and Sprague (1945). They made all possible combination of single crosses (45 in number) between 10 inbred lines. They evaluated the average performance of the single crosses and the inbred lines in yield trials and computed the optimum number of the lines that can go into the synthesis of the most productive synthetic variety. The actual predicted performance of the $F_2$ generation of

the various combinations of inbred lines making synthetic varieties have been given in Table 10.4.

TABLE 10.4

**Calculated yield of $F_2$ generation of synthetic varieties with different number of componental inbred lines (After Kinman and Sprague 1945).***

| *Number of inbred lines* | *Yield in q/ha* | |
|---|---|---|
| | *$F_1$ mean performance* | *Expected $F_2$ yield* |
| 2 | 65.4 | 43.8 |
| 3 | 62.6 | 51.0 |
| 4 | 62.9 | 53.2 |
| 5 | 61.4 | *54.8* |
| 6 | 59.8 | *54.8* |
| 7 | 58.1 | 53.0 |
| 8 | 56.6 | 52.2 |
| 9 | 55.5 | 51.6 |
| 10 | 53.6 | 50.1 |

* *Va ues adapted in q/ha from bu/acre.*

From the data given in Table 10.4 it could be visualised that the optimum performance of $F_2$ performance (54.8) would be obtained by a combination of 5 to 6 inbred lines which are good combiners *inter se*. In a similar way, the number of parental lines optimally needed in the synthesis of a synthetic variety may be determined. It is generally viewed that the best synthetics would result from the use of 4 to 6 inbred lines which are as productive as possible and are good combiners *inter se* (Wellhausen 1952).

(iv) **Making experimental synthetics**

After the prediction of the performance is made, the next step would be the making of the experimental synthetics by mixing the equal quantity crossed seed of all possible single crosses between the chosen componental lines. The plants grown from this combination of crosses from inbred lines are known as Syn. 1 generation. Random pollination of Syn. 1 in isolation produces Syn. 2 and so on. After stabilising the performance for one generation the synthetic population are experimentally evaluated for their performance over years and locations. Usually a period of 2 to 3 years of testing and about 10 to 15 locations are good enough for the release and the recommendation of the synthetic population for commercial cultivation in the farmers fields.

3. **Synthetic varieties in various crops**

The development and the use of synthetic varieties was first proposed by

Hayes and Garber in 1919. Sprague and Jenkins (1943) suggested that synthetic varieties in maize may be useful as the temporary substitute for the double crossess and better serve as the reservoir of desirable gene pools. Hayes, Rinke and Tsiang (1944) made all possible crosses between 20 genetically diverse inbred lines and made up a synthetic from 8 inbred lines that had given the best hybrid performance in single crosses and observed that it gave similar performance to the double cross hybrid (Minhybrid 403) Table 10.5.

TABLE 10.5

**Performance of 8 lines synthetic variety, Minhybrid 403 and open pollinated variety (After Hayes *et al* 1944)***

| *Sl. No.* | *Varieties* | *Yield in quintals per hectare* | *Percentage of Minhybrid* |
|---|---|---|---|
| 1. | Synthetic variety | 46.5 | 102 |
| 2. | Minhybrid 403 | 44.6 | 100 |
| 3. | Open pollinated variety | 37.3 | 83 |

* *Values adapted in q/ha. from bu/acre.*

However, the synthetic variety was found late in maturity time and therefore was not considered equal or significantly superior than the Minhybrid 403. It was however much better than the open pollinated variety not only in yield but also in many other agronomical characters. Lonnquist and McGill (1956) developed a number of promising synthetics using early testing and recurrent selection programmes. Taking the Krug 1 as the base population (100 % performance), the performance of Krug Syn. 2 was observed to be 122%. Results obtained from the 4 first cycle synthetics were carried through from Syn. 2 to Syn. 5 by growing each as an isolated field of 5000 to 10000 plants and out of those selecting only 150 to 250 desirable plants. The average yield of these synthetics from Syn. 2 to Syn. 5 were 100, 108, 111 and 108 respectively in percent of Syn. 2 taken as 100.

Kehr, Graumann, Lowe and Gardner (1961) have reported the forage yield of lucerne synthetics from Syn.1 to Syn.4 generation obtained from 5, two clone synthetics and 14 multiple cross synthetics each made up of 4 to 6 clones. The study revealed that the Syn. 1 had the highest yield. The respective values were 4.46 tonnes/ha. in two clone synthetics and 4.43 in multiple clone synthetics. The average performance of six check varieties were 4.16 tonnes/ha. Several synthetic varieties have been developed and are now in commercial usages in sunflower, bromegrass, crested wheat grass, timothy and white clovers (Briggs and Knowles 1967).

## B. BREEDING COMPOSITE VARIETIES

### (i) *Basic Concept*

In maize breeding programmes, usually the collection of varieties and similar other heterozygous populations are so large that it becomes practically very difficult to make all possible crosses among all the varieties available in a collection and to determine their combining ability individually. However, it has been observed that crossing between certain varietal or interracial populations give very high heterotic response and their yield levels are comparable to some of the best double cross hybrids. It has been generally observed in interracial hybrids that where hybrid vigour occurs, it usually persist in parts through an indefinite number of subsequent generations (Mangelsdorf 1952). Based on some of these observations, the high yielding $F_1$ s of intervarietal or interracial crosses could be advanced to $F_2$, $F_3$, and $F_4$ etc., and in those cases where there is practically very little decline in yield levels in advanced generations could be used as commercial variety by the farmers. It was reported by Dr. Dhawan at IARI New Delhi that crossing among a number of exotic germplasmed with local varieties or varietal complexes as top cross, a number of them gave yield equal to or even better than the best double cross hybrids. Heterotic responses were as high as 20 to 30 per cent or more than the better parent. Further trials with certain $F_1$ populations showed that some of them give little decline in $F_2$ or later generations (Table 10.6 and 10.7).

TABLE 10.6

**Grain yield (kgs/ha) of maize composites (3 generations) at 15% moisture (Northern plains)***

| *Entry* | *Generations* | | | *Percentage of $F_1$* | |
|---|---|---|---|---|---|
| | $F_1$ | $F_2$ | $F_3$ | $F_2$ | $F_3$ |
| *Composites* | | | | | |
| ($B_1$ × Cuba II J) | 4217 | 3433 | 3043 | 81.4 | 72.1 |
| ($J_1$ × Cuba II J) (Sona) | 3447 | 3290 | 3827 | 95.4 | 111.0 |
| (Puerto Rico Gr 1 × Cost Trop. Flints) | 3577 | 3907 | 4330 | 109.2 | 121.0 |
| *Hybrids* | | | | | |
| Ganga 5 | 3577 | ... | ... | ... | ... |
| Ganga 101 | 3613 | ... | ... | ... | ... |

**According to Progress Report of Coordinated Maize Breeding Scheme 1966 ICAR, New Delhi.*

The genetic analysis of yield and other quantitative characters in some of the composite populations has provided data which shows that the additive gene effects play a major role in the expression of these characters. As given in Table 10.6, in certain cases, there was an even improvement in the yield

TABLE 10.7

**Grain yield (kgs/ha.) of maize composites (4 generations) at 15% moisture (Northern plains)***

| *Entry* | *Generations* | | | |
|---|---|---|---|---|
| *Composites* | $F_1$ | $F_2$ | $F_3$ | $F_4$ |
| ($B_1$ × Antigua Gr.I) | 4870 | 4145 (85.0) | 4493 (92.3) | 3687 (75.7) |
| (Kisan) (J1 × Cost. Trop. Flints) | 4376 | 4206 (96.1) | 3808 (87.0) | 3936 (90.0) |
| Jawahar ($A_1$ × Antigua Gr. I) | 4408 | 4155 (94.3) | 4077 (92.5) | 4207 (95.4) |
| *Hybrid* Ganga 3 | 4694 | ... | ... | ... |

* *According to Progress Report of the Coordinated Maize Breeding Scheme 1966 ICAR, New Delhi (Values in parenthesis are percentage of $F_1$).*

performance in the advance generations. This could probably be attributed due to the possible breakage of certain undesirable repulsion phase linkages. Based on this basic concept, a number of composite populations were developed and tested over various locations in India. It was also observed that the composites usually possess a wider adaptability and more buffering capacity to the changed environmental conditions. In country like India where the maize is grown over varied environmental conditions, the seed costs are relatively high and the distribution process is combersome, the composite varieties offer a way out and fit well to immediately boost to the yield level and replace the open pollinated varieties. However, for meeting the needs of progressive farmers, for intensive cultivation for still higher yields, uniform plant types for mechanical harvesting and uniform quality considerations for industrial purposes, the composite will make way for the hybrids. This way the more progressive farmers may take advantage of the higher yielding capacity of the hybrids while the less progressive ones could still be benefitted by planting the composites. As a result, much wider use of the improved seed could be obtained immediately and for the long range, the demand for hybrid seed could be increased gradually by development of better hybrids.

(ii) *Steps involved in the development of composite varieties*

The various steps involved in the development of composite varieties include the(1) making of $F_1$ varietal or racial crosses (2) evaluation of $F_1$ performance (3) evaluation of $F_2$ and $F_3$ composite populations Plate 10.1 (4) then of $F_2$,$F_3$ & $F_4$ and finally the release of composite variety Plate 10.2. Further selection and improvement of the same could be obtained by following a recurrent selection programme *i.e.*, by (1) selecting and selfing a number of desirable plants in the released composite; (2) testing the selection; (3) reconstituting the population and finally evaluating the reconstituted and the original composite population. The detail of the steps involved in the breeding of composite variety as given by Dhawan (1965) are as follows :

Step 1. Screen genetically diverse germplasm collection of base varieties (a) for adaptation by multilocational test and (b) identify the sources possessing resistance to the prevailing diseases and pests and possessing other desirable agronomic attributes.

Step 2. Make all possible crosses among all the suitable varieties of diverse origin or top cross a varietal complex of screened based varieties.

Step 3. Conduct multilocational test with the $F_1$ and $F_2$ generation of the varietal crosses. For further breeding work utilize those combinations that give high yield (as compared to check), high heterotic response comparable to best double cross hybrid released, least decline in $F_2$ and also those that confirm to the norms of other agronomic characters.

Step 4. Evaluate certain of $F_2$ populations which have performed well in Step. 3 and which have the necessary potential to serve later as the composite varieties along with their $F_1$, $F_2$, $F_3$ and $F_4$ populations.

Step 5. Release the best performing composite for commercial cultivation. However, to improve it further subject the best population to recurrent selection. Select a number of desirable plants from this base population.

Step 6. Test the performance of the selections in elaborate yield trials.

Step 7. Reconstitute the composite from the promising selections obtained from the Step 6 and open pollinate it in isolation for one generation.

Step 8. Evaluate the reconstituted and original composites and repeat the recurrent selection so long as significant gains are obtained.

(*iii*) *Genetic improvement of composite populations*

The data in the literature indicate that mass or recurrent selection on an average usually give a gain of 5 to 15 per cent of the base population. Under the recurrent selection programme (Step 5 to 8) a stage will come when the

yield level of composite will pleateau off thereby indicating that the additive genetic variance has been exhausted and no further advancement in yield through selection is possible. It would then be necessary that crossing and introgressing of the desirable gene pools from the new productive and promising germplasm is done to funnel in the new favourable genes to push them for higher level of production. By crossing unrelated varieties with some of the released composites, $F_1$ complexes have been obtained which give 10 to 15 per cent more yield.

Simultaneously apart from only capitalising on additive genetic variance, the non-additive genetic variance where possible and prevalent could be exploited for the development of double cross hybrids. Yield level of both the composites as well as the double cross hybrids could be pushed higher and higher with the introduction of new germplasm at various level of improvement. Some of the composite varieties released in India are low in lysine and methionine amino acids and are usually poor in nutrition. Two genes Opaque 2 and Floury 2 which have recently been discovered as rich sources of high lysine and methionine have been incorporated and as a consequence of this effort three composites namely Protina, Rattan and Shakti have been released for commercial cultivation in India.

(*iv*) *Composite varieties in maize*

The breeding of composite varieties of maize in India, Africa and Mexico has recently demonstrated that certain combinations are capable of giving significantly higher yield than the local varieties and are more or less comparable to the hybrids. It appears now possible that a breakthrough in maize production might come through a fuller utilization of the germplasm pools of favourable genes (Singh, Singh and Dhawan 1964-67, Leakey 1970) and an ambitious programme undertaken in India and elsewhere has given good results. In 1967, six high yielding composite varieties namely, Amber, Vijai, Jawahar, Kisan, Sona and Vikram were released for commercial cultivation. Some of these composite varieties were also tried in countries neighbouring to India. As a result of elaborate testing Vijai has also been released in Nepal and Pakistan. Indonesian Maize breeders have developed two composite varieties, Bogor Composite 102 and Wonosobo Composite, which have performed very well (Subandi 1967). In Africa, Kenya has recently initiated a composite breeding programme. The composite population obtained by a cross between Kitale Synthetic II and EC 537 has already gone in the commercial cultivation. Further improvement of this composite is under way (Ogada, Harrison and Eberhart 1967). Breeding programmes for the development of composites in maize are now currently under progress in India, Mexico, Pakistan, Phillipines and Japan.

(*v*) *Maintenance of the performance of synthetic and composite varieties*

Usually the synthetic and composite varieties according to the genetic theory

are expected to maintain their performance in advance generations under random mating. However in field conditions, it is usually seen that these varieties deteriorate in their performance after 3 to 4 years. To obtain constantly good performance, the seed must be replaced after the third year. Theoretically speaking, according to Hardy-Weinberg Law (1908) there would be no decline in the performance of the populations if there is (1) complete random mating, (2) no mutation, (3) no migration or (4) no selection pressure or selective fertilization. If the gene frequency remains constant from generation to generation, if the sampling size for the foundation and nucleus seed multiplication is adequate, there is no unequal viability of different gametes, there is no attack of pathogen (diseases, insect pests, etc.) no mechanical mixture with the seed of other varieties, no faulty or patchy germination in field, no poor agronomy, then the performance of the composites and synthetics will remain constant. These conditions though theoretically desirable but actually are not met with in toto under the field conditions. The synthetic and composite varieties as a consequence, therefore, deteriorate in their performance. The forces of natural or artificial selections, all the more, modify the performance of the varieties from generation to generation.

The estimated performance of the synthetic variety as has been given in Wright's formula depends upon the mean performance of $F_1$ and a correction factor in terms of $(F_1\text{-}P)/N$. If somehow the value of P is increased the estimated performance could be enhanced. It has been generally suggested that the performance of the synthetic and composite varieties could be maintained or even improved provided proper selection of desirable plants is practised in the parental inbred or varieties, and adequate sample size is taken for maintaining the commercial seed stocks, proper isolation distance is kept in the seed production programme and an adequate care is taken so that the seed and the genetic purity are maintained and the standard agronomic practices are followed in the production of the commercial seed. Apart from this, the original inbred lines or varietal base populations that go into the synthetic variety may be maintained separately and carefully so that the released synthetics or composites may be further constituted when needed. When the synthetic or the composite varieties are constituted, the combination of the base populations may either be kept constant or at times be changed slightly by substituting an improved version of the base population in making the composite. This will maintain or even improve the performance of the original synthetic or the composite variety.

SECTION FIVE

# GENETIC IMPROVEMENT OF HYBRIDS

# 11

# Population Improvement in Hydrid Varieties

Population improvement is one of the major aspects of scientific crop breeding. The basic concept of this type of improvement is to accumulate, infuse and improve the frequency of favourable genes in an otherwise established population. This usually includes the removal or substitution of an undesirable gene with a counterpart favourable one with the superior performance in a given environment, in-corporation of a balanced gene combination for better performance *per se* or may be even bringing in a better plant type congenial for better physiological activity and biochemical versatality resulting in the better performance of a population.

As pointed out by Hayes (1963), it seems probable that most of the commercial hybrids could be improved in one way or the other for one or more of the agronomic attributes. This is primarily because some of the lines in each hybrid generally excel in comparison with the other in their ability to transmit desirable characters to their hybrid progeny and thus provide scope for the improvement of the weak inbred lines of the hybrid to further better its performance.

The choice of the particular population improvement technique chiefly depends upon the genetic informations. Since by and large, the population improvement techniques envisage controlled crossing followed by selection and recrossing followed by further selection, this information is vital. Therefore, before deciding upon the choice of a particular methodology, such information as the type of gene action involved in the expression of the attribute under improvement, the nature of the genetic associations, extent of heritability, the various possibilities of the genetic manipulations should be available. Some of the procedures which could be successfully utilized in the population improvement of parents used in released hybrid populations may be enumerated as the utilization of backcross method of breeding, convergent improvement, gamete selection, technique for the removal of complementary genes, step ladder method of breeding and incorporation of dwarfing genes in the commercial tall hybrids etc. A brief description of the method are as follows :

*(ii) Convergent improvement*

Convergent improvement is a system of double backcrossing for the purpose of improving each of the two inbred lines of a cross without much interfering, changing, or modifying the combining ability of the cross. As this method basically utilizes the system of backcrossing, it basically improves the inbred lines and betters the performance of the cross by accumulating the favourable gene(s) obtained from the counterpart lines by careful selection following each backcross and by discarding the less favourable genes.

This procedure was first suggested by Richey (1927). It has been discussed and supported by the experimental evidences obtained by Richey and Sprague (1931), Murphy (1942), Hayes *et al.* (1946) and Lonnquist (1960). The method is based on the premise that if one selects a good single cross (A × B) and assumes that in the expression of the heterosis in $F_1$ results as a consequence of the accumulation of favourable, partial or complete dominance of genes obtained from the two componental lines, then by simultaneously improving the two inbred lines A and B into the inbreds A′ and B′ by double backcrossing *i.e.*, A with B and B with A, the hybrid would be theoretically improved. This is because during the process of backcrossing and selection, the desirable dominant favourable genes in which line A is lacking would be obtained from line B and they would be accumulated in line A and vice-versa in B from A. Therefore, if the partial dominance of linked factors or the dominance theory to explain heterosis holds good, the recovered line would perform better. When these improved lines are again crossed, the resultant hybrid would be superior to the original single cross (A × B) in performance with respect to the characteristics under selection. The recovered inbreds A′&B′ would theoretically be more desirable as compared to original A and B. This is because in A′ and B′, most of the undesirable recessive genes limiting the vigour and productivity of the line might have been shed during the process of backcrossing and replaced by counterpart dominant favourable genes obtained from the counterpart line of the cross.

The various steps involved in the convergent improvement as given by Hayes *et al.* (1955) are as follows :

Step 1. Select a high yielding desirable single cross.

Step 2. Backcross the $F_1$ to both of the parents and then further backcross for 4 - 6 generations simultaneously in the two series to the respective inbred parents. Select only the vigorous plants which have desirable characteristics needed to be improved during the period of backcrossing and use only these in making the backcrossing.

Step 3. Self the backcrossed lines after 4 - 6 generation of backcrossing and select within the selfed lines.

Step 4. In order to obtain further improvement, repeat the above steps (2 to 3) with the recovered inbred lines.

(i) *Utilization of backcross method of breeding*

Backcross method of breeding could be conveniently utilized for the incorporation of a specific gene or genes systems governing the characters like resistance to diseases and insects, physical and chemical quality characteristics, male sterility and fertility restorer genes etc. in the componental inbred lines of an established hybrid with a view to improve its performance and make it better fitting for the production programmes. Suppose if a particular single cross, three way cross or double cross hybrid suddenly becomes susceptible to a particular pathogen or pest, then to improve the hybrid for further commercial production, resistance to the disease may be incorporated through a standard backcross breeding programme. The various steps involved in the incorporation of resistance to a disease say for example-leaf blight in a double cross $(A \times B) \times (C \times D)$, would be to grow the double cross hybrid and its inbreds under artificial epiphytic of leaf blight and then to identify the componental inbred line of the double cross which contributes to the susceptibility to the disease (say line A). Then the next step would be to cross this genetically susceptible line A with a donor inbred line (say F) possessing the source of resistance to leaf blight and backcross as $(A \times F) \times A$. If the gene governing resistance to leaf blight is dominant, then select the resistant plants in backcrossed progenies and again backcross with A. If the gene governing resistance is recessive in nature, then self the backcross progeny to identify the resistant plant and again backcross it with the recurrent line A. Repeat the backcrossing for 4 to 6 generations depending upon the non-adaptability attribute of the donor source F, and finally recover most of the genetype of line A. Designate it as A′. Reconstitute the double cross hybrid as $(A' \times B) \times (C \times D)$ and compare its performance with the original double cross hybrid $(A \times B) \times (C \times D)$.

Hayes *et al.* (1955) reported the utilization of the backcross method of breeding for the incorporation of smut resistance in a three way cross maize hybrid Minhybrid 301. The pedigree of this hybrid was $(11 \times 14) \times$ B 164. In this hybrid, it was found that the male inbred line B 164 was susceptible to the smut disease. The early maturing inbred line C 37 was used as the donor parent for transferring resistance to smut through the standard backcrossing programme. Genes for resistance to other diseases, insects, lodging, shattering, drought resistance etc. may also be likewise incorporated in the componental inbred lines to improve the hybrids.

In maize, sorghum, bajra, onion, wheat hybrids, backcrossing has frequently been used for the incorporation of male sterility and fertility restorer gene system. The same has been described in Chapter 9.

Recently backcrossing technique has also been used for the incorporation of genes for certain quality characteristics, various plant type attributes etc. in the population improvement programmes of the hybrid varieties.

The initial experimental data supporting the value of this method was first given by Richey and Sprague in 1931. These workers suggested that 3 or 4 backcrosses followed by 2 or 3 generations of selfing produced inbreds that were somewhat superior to the original inbreds. The data obtained by Murphy (1942) provided additional support to this theory. He observed the performance of the original and recovered lines of the two single crosses ($C_{15} \times C_{19}$) and ($C_{16} \times C_{20}$). The recovered lines were obtained by two, three or four years of double backcrossing followed by selfing for two years. Test crosses were made of the type (recovered $C_{15} \times C_{19}$), and (recovered $C_{19} \times C_{15}$) for each single cross group. Fifty one such single crosses were compared with respective originals in yield trials. Under the conditions of the experimental trials, two crosses were, however, distinctly higher in yield than the original single crosses used. The study indicated that the yield of $F_1$ crosses, apart from other desirable characteristics themselves could be increased by this method of improvement.

Hayes *et al.* (1946) reported the utilization of this method of population improvement for the double cross maize hybrid Minhybrid 403 (11 × 14) × (C23 × A374) In this double cross, the inbred lines 11 and 14 were early in maturity then C23 and A374 but were found susceptible to smut and lodging behaviour. After 2 - 3 generations of backcrossing and selfing, the recovered lines of (11 × C23) × 11 and of (14 × A374) × 14 were earlier in maturity and also comparatively better in yield performance. The double cross, obtained from the recovered lines were compared with the original Minhybrid 403 and several of these performed significantly better than 403. They however, suggested that the improvement of an inbred line could be accomplished without modifying the specific combining ability of the cross rather easily by convergent improvement provided the inbred line of a cross has particular weaknesses for a character(s) and the other inbred carries these (Hayes, 1963).

Lonnquist (1960) utilized convergent improvement for the genetic improvement of the single cross hybrid (Wf9 × 38-11). This single cross is quite desirable for seed quality and expresses good heterosis. The objective of improvement of this cross was primarily to improve the pollen shedding ability of Wf9. The performance of double crosses where the original single cross (Wf9 × 38-11) was used as parent and 5 double crosses obtained from recovered lines were compared in elaborate yield trials. He reported that in all 5 comparisons, the double crosses made from the recovered lines yielded more than the original double crosses, where original single cross (Wf9 × 38-11) was one parent. However, the former, were having somewhat higher moisture content than the latter.

Though discovered long before and experimentally proved useful, convergent improvements has not been frequently practiced in the modern plant breeding programmes. This is because the yield improvement obtained by this method have not been either substantial or spectacular as the

convergent improvement is not very effective to improve the yield *per se.* Theoretically speaking also, there has not been exclusive proof to the fact that the dominance theory of heterosis, upon which this method is primarily based, is universal and works well in all the hybrids needed to be improved Lastly, the rate of genetic gain is rather slow The plant breeders, then usually prefer to altogether replace a hybrid by a new one available rather than to improve the old hybrids at a comparatively slow rate of genetic improvements of yield. This method, would, however, be useful under the situation where a particular hybrid is rather hard to beat and it would perhaps better be corrected advantageously by this method of population improvement.

*(iii) Gamete selection*

Gamete selection is a method of selecting and combining desirable gametes from a genetically variable heterozygous population into the background of an inbred line of known performance and combining ability. In the improvement of hybrid populations this is done primarily to improve the desirability of a particular weak inbred line which is otherwise good. The basic procedure of this type of selection essentially involves the crossing of a good inbred line of known performance with a random sample of pollen from an open pollinated variety or any other known heterozygous source, self pollinating the individual $F_1$ plant obtained from the variety × inbred cross, simultaneously topcrossing them to an appropriate tester stock. This is followed by conducting a yield trial of the inbred × variety cross progenies, selecting the better performing progenies of the crosses and then finally, continuing selection with the selfed seed of the original $F_1$ seed.

Stadler (1944) suggested this method for the first time. This was primarily for obtaining and improving the inbred lines. He emphasized that a good gamete occurs much more frequently than an equally desirable zygote from a random population of a variety Therefore, supposing if the superior zygotes occur in a heterozygous base population, with a frequency of $P^2$, superior gamete would occur in much higher frequency of P. In other words, if the frequency of a desirable gene in a population is numerically 0.01 *i.e.*, one in one 100, then the probability of obtaining a desirable zygote would be $0.01 \times 0.01 = 0.0001$ or 1 in 10,000. Therefore, the gametic sampling would offer a more efficient means of sampling open pollinated varieties for obtaining good inbreds than through the standard inbreeding procedure.

Hayes (1963) has detailed the procedure and use of this method in relation to the improvement of an inbred say for example A in a double cross (A × B) × (C × D). Supposing that the inbred A is inferior to B and it gave lower yield than B in crosses with (C × D). Taking V as the source of desirable gametes from a highly heterozygous but adapted variety, he outlined the following procedure for this method.

Step 1. Pollinate A with the mixed pollen obtained from the desirable plants of variety V selected as a source of superior gametes.

Step 2. Self pollinate selected individual plants from these crosses and use the pollen of each individually to pollinate plants of (C×D) to obtain crosses with (C×D).

Step 3. Test the crosses produced by Step 2 in elaborate yield trials and study all the characters of importance including yield, in comparison with crosses of A (C×D).

Thereafter, either of the two methods of further selection could be followed. Firstly, the selection in selfed lines obtained from the original selfed plant from the step 2, which have performed very well in topcrosses with (C×D) single crosses. The other alternative selection may be that about 10 or so best performing original selfed plants from step 2 may be mixed together to constitute and produce a new population. From this population, a second cycle of gamete selection may be started after one generation of open pollination to ensure recombination of genetic factors. Selected plants from this population may be handled as in step 2 and 3. Finally, after two or 3 cycles of selection for superior plants through recurrent selection from this population, superior lines may be isolated to take the place of A in the double cross (A′×B) × (C×D). Finally, this may be compared with original double cross hybrid (A×B) × (C×D).

Gamete selection is essentially a method of early testing. It has been indicated that it may be useful for selection and isolation of inbred material from the open pollinated varieties (Pinnell, Rinke and Hayes, 1952; Lonnquist and McGill, 1954).

(*iv*) *Removal of undesirable complementary genes*

Complementary genes are genes which individually govern the expression of the same character but when they come together in a hybrid, they interact to produce a distinct and different expression. A typical example of this has been the genes governing white flower colour in peas. When the two white coloured flowered varieties with $A_1A_1a_2a_2$ & $a_1a_1A_2A_2$ genotypes are crossed together they produce a hybrid ($A_1a_1A_2a_2$) with pink colour. In Indian maize breeding programme, the hybrid Ganga Safed Makka No. 2 was released for commercial cultivation during 1963. This is a flinty, white grained stable and high yielding double topcross and is commercially popular with the maize growers. However, this hybrid has one undesirable seed attribute. In the double top crossed seed progenies, some pinkish blue grain appear in varying number. This, though, not affects its yield performance, however, it impairs its seed quality for marketing purposes.

The genetic analysis for the appearance of the pink grain in this double top cross has shown the presence of the complementary genes. These genes are located both in the white grain single cross (Tenn 29×Eto PLBL) and the

white grain variety Rudrapur local. The possibility of xenia being the reason for this, has, however, been excluded. The breeding scheme which could be effectively followed for the removal of such complementary factors from a double topcross hybrid has been given as follows :

Step 1. Self pollinate a number of plants (250—400) in the male parent of double topcross *i e.*, in the open pollinated variety (V). Number them serially and simultaneously cross them individually with the single cross female parent (A×B).

Step 2. Grow these crosses individually the following crop season. Self them. Examine the grains set on the individual ears of the plants. Select only those plants which does not show the pinkish blue grains.

Step 3. Go back to the self seed obtained in step I of the selected plants and reconstitute the variety from the selected plants Compare the performance of original (V) and the reconstituted (V′) variety.

Step 4. Reconstitute the double topcross as (A×B) × V′ if their yields are equal and compare its performance with the original double topcross hybrid (A×B) × V.

This is a typical model and could be successfully followed in the removal of the undesirable complementary genes. The procedure has been followed for the removal of the pinkish blue grains from the double topcross hybrid Ganga Safed Makka No. 2 and the appearance of the pinkish blue grains in this hybrid has been considerably reduced.

(*v*) *Step ladder method of breeding*

Dhawan (1965) has suggested a step ladder method of breeding for a phased elimination of inferior inbred lines and by the substitution of an altogether new inbred lines for further genetic improvement of an established double cross hybrids in maize, say for example (A×B) × (C × D). This method involves the a) making a number of three way crosses *i.e.* (A×B) × E, F, G, H, etc. by using the better parental single cross (A×B) or say (SC1), as a topcross tester to screen the new inbred lines (E, F, G, H etc.) emerging in a breeding programme. This is followed by the identification of the superior specific combining inbred lines from the three way cross test for evaluating them in a replicated yield trial. The further step which follows is the prediction of the performance of a new double cross. In this the tester SC1 forms one parent and a new single cross SC2A (E×G) is developed from two of the superior lines E and G. The superiority of lines E and G has already been established by the three way cross test. It forms as one of the parent in the new double cross. In this way the inferior single cross (C×D) is replaced by a new and superior single cross (E × G) and the new double cross hybrid (A × B) × (E × G) is developed. This new hybrid not only gives the better yield than the original double cross

but is also usually agronomically superior. The data given in Table 11.1 with respect to the population improvement of a released double cross hybrid Ganga-3 illustrate the point clearly.

TABLE 11.1

**Yield performance (kgs/ha) of a released double cross hybrid Ganga-3 (After Dhawan 1965)**

| *S.No.* | *Pedigree of the entries* | *Yield (kgs/ha)* |
|---|---|---|
| 1. | AdeC-A406 × Tester (SC1) or (A×B) E | 5268 |
| 2. | G 101-A37×Tester (SC1) or (A×B) G | 4660 |
| 3. | Predicted double cross (A×B)×(E×G) | 4964 (20% increase) |
| 4. | Original Ganga-3 Hybrid (A×B)×(C×D) | 4120 |
| 5. | SC1 Tester (A×B) | 4323 |

As given in Table 11.1 single cross (A×B) of double cross Ganga 3 was used as the topcross tester on the new lines AdeC-A 406 (*i.e.* E) and G 101-A37 (*i.e.* G). The performance of the new double cross (A×B)×(E×G) was predicted to give 20 per cent more yield than the original Ganga 3. The performance of the predicted double cross would be experimentally verified under field condition and if it gives the actual yield as per prediction, it could be released to replace the original Hybrid Ganga-3.

Further, improvement of this improved double cross (A×B)×(E×G) if needed could be accomplished by following a second cycle of step ladder method. In this cycle, (E×G) is used as a tester and (A×B) is replaced by some of the newer and promising inbred lines developed in the inbred breeding programme.

(*vi*) *Breeding dwarf hybrids*

With the successful incorporation of dwarfing genes in wheat and rice to breed lodging resistant, fertilizer responsive varieties, it has now been considered profitable to breed dwarf hybrids in other cereals where lodging is a serious problem. Todate, the incorporation of dwarfing genes to breed lodging resistance varieties has already been accomplished in sorghum and bajra hybrids. Some of the dwarf hybrids in these crops are already under commercial production. The breeding efforts are now under way to produce lodging resistance dwarf hybrids in maize.

In maize, it is generally believed that the dwarf plant of the hybrids will provide mechanical resistance to lodging (Leng, 1960; Lonnquist, 1960), and breeding dwarf hybrids in this crop would be helpful in solving the problem

of lodging and the yield loss. In India, presently lodging is a serious production problem, especially in Uttar Pradesh. The average reduction of grain yield due to this has been reported to be 22 per cent (Asnani and Paliwal, 1966).

(*a*) *Dwarfing genes in maize*

In maize, 15 genes have been reported to reduce the plant height (Table 11.2). However, three types of dwarfs are generally met with and are important from breeding point of view. They are true dwarf (rd), compact dwarf (ct) and brachytic dwarfs (br2). The true dwarfs are highly abnormal in appearance. Compact dwarf plants are reduced proportionally in various plant parts. The brachytic dwarfs are those in which the plants are normal except for the shortening of the internodes below the ear. Of the various dwarf genes, the last one has been reported to be intensively studied and extensively used for the development of short stalked hybrids in maize (Stein, 1955; Scott and Campbell, 1969; Leng, 1957 and Anderson and Chow, 1963).

(b) *Studies with incorporation of br2 gene in maize hybrids*

Leng (1957) for the first time reported the development of dwarf version of double cross hybrid US 13 by incorporating br2 gene in all the four

TABLE 11.2

**Symbols and brief description of 15 dwarfing genes in maize**

| *S.No.* | *Character* | *Symbol* | *Chromosome on which located* | *Brief description* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| 1. | Brachytic | br1 | 1 | Stalk internode shortened below ear. Plants 1/4-1/2 normal in height. |
| 2. | Brachytic | br2 | 1 | Plant dwarfed by about 40% reduction in internode length. No effect on upper most internode and peduncle. |
| 3. | Brevis | bv1 | 5 | Plant half normal in height due to internode shortening. |
| 4. | Clumped tassel | ct | 8 | Semi-dwarf, tassel branches fused. |

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| 5. | Dwarf plant 1 | d1 | 3 | Plant very short w ith broad leaves, stamens develop on ears, staminate inflorescences compact, reversible by gibberlic acid. |
| 6. | Dwarf plant | d2 | 3 | Similar to d1, Gibberlic acid reversible, usually sheds no pollen. |
| 7. | Dwarf | d3 | 9 | Dwarf, usually male sterile gibberllic-reversible. |
| 8. | Dwarf | d5 | 2 | Gibberlic-reversible dwarfing. |
| 9. | Dwarf | d8 | 1 | Dominant dwarf, not responsive to gibberlic acid. |
| 10. | Nana dwarf | $na_4$ | 3 | Plants 1/4-1/3 normal height leaves, short, stiff and twisbed. |
| 11. | Pigmy | py | 6 | Plant short with thick stiff leaves, ear production is poor. |
| 12. | Reduced | rd | – | Dwarf plant. |
| 13. | Small plant | spl | 6 | Dwarf plant. |
| 14. | Thick tassel dwarf | td | – | Plant strongly dwarf including ear and tassel. |
| 15. | Yellow dwarf | yd | – | Dwarf virescent. |

parental inbred lines. On the basis of three years of tes ting of single cross brachytic hybrids, Leng (1958) (1960) stressed that the hybrids prosses sed considerable promise for lodging resistance. However, they yielded approximately 20 per cent less than their normal counterparts. This was probably due to the fact that the recovery of the original genotype of parental inbreds were not completely achieved by fewer number of backcrossing (2—3). Anderson and Chow (1963), however, reported that the incorporation of br2 gene in a single cross (M14×Oh 43), out of the three single crosses viz., (M14×Oh43), (Hy2×L317) and (111×AW 22) gave 5146 kgs/ha while the yield of its normal counterpart was 4131 kgs/ha. In the remaining two single crosses, the dwarf version did not contribute to any additional yield. They attributed the variable effect of br2 gene in the three single crosses to the genes linked to br2 and the occasional recombination between them as well as to the pleiotropic effect. Usually the pollen shedding and silking time were delayed in the dwarf hybrid as compared to their normal counterpart.

Campbell (1966) incorporated br2 dwarfing gene in 100 inbred lines by backcrossing. He reported that the dwarf hybrids yielded more or less equal

to their normal sized counterparts. However, lodging resistance was much better in the former than in the latter lines.

Chutkaew (1969) studied the comparative performance of the dwarf and tall version of 5 inbred lines and 4 crosses of maize involving br2 gene. He reported that on an average, the dwarf version yielded significantly higher than their normal counterparts. They also had better stem thickness, lower plant height and less percentage of stalk breakage and lodging as compared to the tall versions. However, he emphasized that the performance of a dwarf version much depends on the genetic background into which br2 gene is introduced and suggested that it is possible to develop high yielding dwarf hybrids with other desirable characters by selecting proper genotype.

It is generally believed that in a crop like maize, too short a plant height might not be much advantageous, a too tall hybrid would also be prone to lodging. Therefore, a medium hybrid *i.e.*, somewhere between 150-170 cms might be desirable one. Keeping this in view, Thompson (1964) compared a group of dwarf, semi-dwarf and normal plants of several genotype. He reported that in the semi-dwarf, satisfactory yield levels could be obtained. Bholanath (1970) studied the effect of br2 gene on yield and other characters in normal and dwarf versions of 5 inbreds and 4 single cross hybrids at two levels of fertility (150N; 60 $P_2O_5$ : 40 $K_2$ O kgs/ha) and (200 N, 60 $P_2O_5$ and 40 $K_2$ O kgs/ha). The results obtained by him indicated that the performance of the two versions depended upon the genetic background in which br2 gene was introduced. The dwarf version yielded at par at lower and high levels of fertility. The dwarf versions, however, had larger leaf area, more ear length, 1000 kernel weight, short plant height Plate 11.1(a) and Plate 11.1 (b) and less lodging. Pandey (1971) studied the effects of 4 sources of dwarfing genes (br2, d1, ct and d3) in 4 base populations of maize viz., CM 201, CM 111, Kisan and Jawahar composite at two locations (Delhi and Pantnagar). He observed that out of the 4 dwarfing gene studied, the br2 had the minimum adverse effect on the yield and other agronomic characters. On the basis of average effects of dwarfing genes, it was concluded that brachytic dwarf versions of CM 111, Jawahar and Kisan under Delhi conditions and d1 dwarf version of CM 201 and the br2 dwarf versions of Jawahar & CM 111 under Pantnagar conditions could be effectively utilized in the breeding programmes to produce high yielding dwarf varieties in maize.

SECTION SIX

# FUTURE PROSPECTS

12

# Heterosis Breeding : Possibilities of the Future

The basic and applied researches of the past have provided the present day plant breeders a working system of hybrid breeding methodology. This is particularly in terms of a reasonable understanding of heterosis, the relations of the inbreds or parents to the performance of the hybrids, techniques for the quick testing for combining ability of the parental populations, a way of the prediction of performances of the hybrid populations, realization of the importance of genetic diversity to heterosis breeding and a basic knowhow for the genetic improvement of the hybrid populations so vital to heterosis breeding. Some of other significant contributions of the past workers in this field have been the systematic organizations of a cooperative research effort, exchange of valuable research ideas and materials through the length and breadth of the countries as well as across the frontier of national boundaries. In most of the agricultural institutions, hybrids have often been used as a tool to demonstrate the power and potency of the scientific plant breeding in extension programmes. All this in the past has provided an impetus in bringing out quick genetic and economic gain through the use of hybrids specially in cross pollinated crops.

The production of hybrids has also given considerable impetus to the improvement of basic technology concerned with the organized production of hybrids and has created the scope for the systematised commercial seed production, processing and distribution. However, some of the significant achievements of the past and the current trends in heterosis breeding indicate that to maintain the tempo of progress in yield improvement through utilization of hybrids, greatly expanded efforts would be needed in future to solve the challenging problems ahead. There is no room for pessimism. However, there is every reason to think that the great record of success in heterosis breeding could be continued, even bettered provided the plant breeders in this area of research keep them at the frontier of knowledge and the developments in the related disciplines,work at their jobs and look ahead in advance. This basically furnishes the background for a brief discussion for the possibilities of the future in hybrid breeding programmes.

The future, in hybrid breeding research may see some of the accelerated

efforts in the areas of tapping new germplasm resources, conversion of self-pollinators into cross pollinators, greater utilization of male sterility, a fuller understanding of heterosis, evaluation of new breeding approaches and techniques, extension of hybrid breeding to new crop areas, restructuring the hybrid populations for better agronomic and biochemical versatality, balanced interdisciplinary cooperations and perhaps use and assimilation of many new ideas for tackling the new problems. A brief discussion of these aspects is as follows :

(*i*) *Germplasm resources*

In most of the crops, it now appears that a yield pleateau has almost reached. The yield levels in maize, sorghum, wheat and rice etc. have increased considerably since the beginning of the century. In the process, a large amount of genetic stocks have been collected, screened and utilized. A vast potential for further collection, and utilization, however, yet remains to be tapped. Therefore, if the heterosis breeding efforts have to be advanced further, exploration of the new germplasm resources has to be launched. Probably a large force devoted fully to this task would be needed in future and a full-fledged establishment would be fully justified. Burton (1966) has rightly emphasized that the future will see greatly expanded programmes for the collection and preservations of germplasms and the world collections may increase in size.

The utilization of computer facility may ease the job of classifying and cataloguing work of a large number of collections. Wherever possible, this facility may also be successfully extended to the prediction of the performance of a very large number of possible double crosses and help quickly pick the productive hybrids.

The vavilovian job of further combing the centers of origin of various economic crop species for valuable germplasm would be needed. With the development of scientific agriculture and extensive use of improved and uniform hybrids or varieties, the much needed local and the wild material with valuable gene pool is continuously being lost. If the same rate is allowed to continue, some day we may wake up to find the genetic variability of our crop plants too small for continued improvements and our priceless heritage of germplasm squandered and dissipated (Harlan, 1966). Therefore, these valuable local and wild populations must be saved, collected and preserved before the time runs out. To a certain extent, a good beginning has already been made by the countries/organizations concerned with the improvement of the specific crop or crops. However, in future the volume of efforts has to be expanded, commensurate with the extent of genetic improvement expected. It would be rather imperative to maintain atleast one breeding population containing almost entire world collection of germplasm by almost each country concerned with the breeding of the crop in which it is intensely and vitally interested. This will help preserve the most

valuable genes which have survived the onslaught of a rigorous selection under natural conditions. These populations so maintained could also have quick natural recombinations and may provide even an opportunity to break undesirable linkages under natural conditions and provide even better germplasm for the future.

(ii) *Self pollinators into cross pollinators*

Considerable range of exploitable heterosis has been reported in a number of self pollinated crops (Chapter 7). But owing to their built in floral structure and breeding behaviour, the situation with respect to free and unrestricted transfer of pollen from one plant to another and consequently the commercial hybrid seed production remains usually complicated and costly. This is a major bottleneck which holds back the exploitation of heterosis in such crops. Search for new genes or gene system which may possibly convert or induce the self pollinating behaviour into cross pollinating one alongwith a mechanism for free and unrestricted natural transfer of pollen from plant to plant would be needed in these crops. Sorghum and wheat are two good examples of such a situation and future may see even much more conversion of such behaviour.

(iii) *Male sterility and hybrid seed production*

Exploitation of heterosis is going to visualize much expanded utilization of male sterility in future than what is now. A good example of the utilization of male sterility and its impact on crop yield in India is seen in the bajra breeding programme. Bajra is an important crop of dry areas of this country and feeds million of people inhabiting these areas. Only after the availability of male sterile lines, it became possible to breed and commercially produce a number of high yielding hybrids in this crop in this country. More or less similar is the story with the sorghum crop in this country.

Cytoplasmic male sterility provides one of the most workable system of the utilization of male sterility for commercial hybrid seed production in crop plants. It has been pointed out by Duvick (1966) that this type of male sterility could be found in almost all the crops species if we look for it.

In future, however to make male sterility more convenient to utilize and perhaps for the reasons of avoiding the search and complications of full fertility restorer mechanisms, some biochemical research for the induction of male sterility through biochemical means would be much needed. Some chemicals which could systematically and selectively affect the pollen formation *i.e.*, microgametogenesis without affecting the egg formation (mega gametogenesis) and be better applied as the seed treatment at the time of planting, could perhaps be the ideal one. This will make the utilization of male sterility much easy and economical. If the researchers could find a effective chemical device, the same could well be extended to plants. The hope of further cutting the cost of the hybrid seeds would greatly rest on such basic discoveries vital to the seed production programme.

(iv) *Understanding heterosis*

Heterosis as it is, is not a very clearly understood phenomenon. Burton and Sprague (1961) have emphasized that the extent and efficiency of hybrid use will depend on a more adequate understanding of the phenomenon of heterosis and related problems in statistical genetics. They further emphasized the importance of the following areas in need of future research viz., the various type of gene action involve in quantitative characters, the magnitude of the genotype × environment interactions, in relation to the estimate of several components of genetic variances and the nature of genetic associations and differences between the varieties.

A good theoretical insight into the causes of heterosis has been provided by the researches of Hageman *et al.* (1967). In nutshell, they have emphasized the integrated and balanced production of essential metabolities materially affect the growth and performance of the attributes. In hybrids, perhaps similar mechanism might be playing an important role in the expression of of heterosis In future, fundamental studies on the mechanics of nature of gene action through the elaborate physiological as well as biochemical techniques may perhaps bring a clear understanding of the phenomenon of heterosis.

(v) *New approaches and techniques*

In future hybrid breeding programmes, far new approaches and efficient techniques would be needed to quickly identify the specific combining lines especially in the initial breeding material. Perhaps, certain suitable marker genes or quick physio-biochemical means like mitochondrial complementation in the early seedling stages if standardised could greatly help the breeders in screening large population and developing far productive hybrids.

An exciting prospect of the heterosis breeding programme would be the possibilities of developing hybrids with broadbased disease resistance. In other words, purposefully superimposing resistance breeding on heterosis breeding. The development of multilineal hybrids has been suggested and demonstrated in wheat (Rodriguez *et al.*, 1967, Fig. 12.1). So far the breeding for resistance to diseases and for increasing the productivity of hybrids *per se* are usually used to tackle them rather separately. However, it is now being felt that at higher level of productivity potential, a weakness of one may considerably upset the improvement in the other. For a balanced and sustained improvement, a proper coordination between the two would be essential. Utilizing this approach, the Mexican and Rockefeller Foundation Wheat Improvement Programmes have developed two stripe rust resistant multilineal varieties Miramar-63 and Bonza-65. These varieties are now successfully being grown in Columbia and Ecuador (Johnson and Schmidt, 1968). The development of these varieties have opened up the possibilities for such disease resistant hybrids for the future.

In maize, especially, where hybrid seed production is a multistage operation, in future, for the economic ease of seed production and perhaps for more uniformity, production of single crosses would be taken up for commercial production. It is now possible to obtain a single cross which is agronomically better than three way or double crosses particularly in sweet and pop maize hybrids.

Much more expanded breeding and genetic investigations would be needed to determine the extent to which it may be possible to improve and incor-

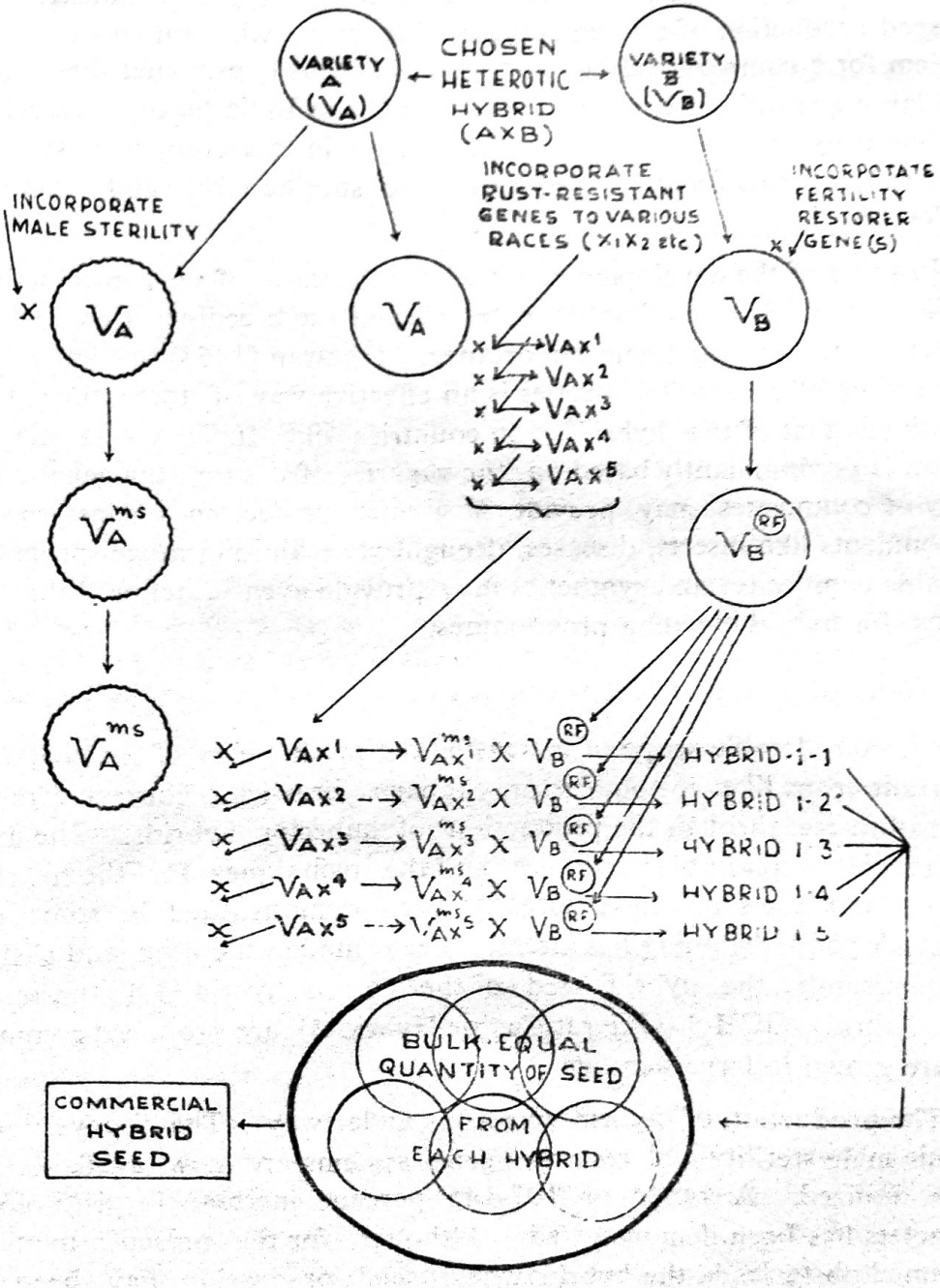


*Fig. 12.1* : Development of multilineal hybrid wheat (After Rodriguez *et. al.* (1967) V=variety, ms=male sterility, Rf=Fertility restorer gene, $X_1$=parent giving resistance to race 1 etc.)

porate balanced protein, oil, carbohydrates etc. while maintaining the high grain yield potential in the hybrids in times to come as compared to those now available. The available literature on the genetic studies reveals that it would not be too difficult to produce hybrids with reasonably good yield and a higher expression of these chemical characters.

In many crops, the need for diversification of the hybrid varieties is being felt. The heterosis breeding programme in future, would see the development of hybrids for special purposes like those for human food, for poultry and livestock feeding, forage production and for various types of industrial usage. The seed production of a large number of hybrids will not pose a serious problem for a comparatively large number of hybrids provided they are needed in large quantities. It is presently being felt in India that the hybrid maize breeding programme will not have much future in this country as such unless the specific hybrids for specific purposes and specific area and crop pattern are developed.

In some of the developing countries for the ease of seed production and distribution, utilization of partial heterosis and the breeding of composite and synthetic varieties would gain importance. Dhawan (1965) has indicated that the breeding of composite varieties is an effective way of increasing the yield potential as that of the hybrids. In countries like India where maize production is predominantly based on the vagaries of nature, the genetic heterogenity of composites may provide a greater protection against the harsh environments like insects, diseases, drought etc. In long range programmes, desirable composites and synthetics may provide even a better source populations for hybrid breeding programmes.

(vi) *Heterosis breeding in new crop areas*

There is considerable scope of increasing the productivity of the economically important crops like, cotton, castor, cabbage, brassicas, linseed, forage legumes, radish etc. through the production of superior hybrids. The extent of commercially exploitable heterosis and the techniques for the hybrid seed production of the same, have already been demonstrated in some of these crops. A good beginning has already been made in cotton and castor. In India, presently, the hybrid seed of the cotton hybrid H 4 and one of the castor hybrids, GCH-3 (Gujarat Castor Hybrid-3) are produced commercially and are grown in large acreage.

The production of hybrid wheat is under way. The discovery of cytoplasmic male sterility and restorer gene systems are now available and are being utilized. A range of 107-146 percent increase in yield and other characters has been demonstrated. Although for the present, many of the presumed obstacles in the hybrid wheat seed production have been solved, several important ones yet remained unsolved. The future of hybrid wheat would depend upon the combination of many of the interacting technological and economic factors. Their future solutions would ultimately decide upon

the economic feasibility of the hybrid wheat on a large scale in times to come.

Enormous possibilities of the commercial exploitation of heterosis yet remains untapped in fruit and vegetable crops. In some of the such crops, builtin sexual and asexual means are available which could be effectively utilized. Cultivated bananas are one good example of the utilization of increased vigour and seedlessness in triploidy form from generation to generation through the vegetative means. In future, the techniques of hybrid breeding could be considerably used in the breeding of fruit crops like, apple, oranges, pears, guava, mango, many others and vegetable crops like tomato, radish and carrot. Good possibilities for the exploitation of heterosis in ornamental plants also exist and the future will see much expanded research effort for hybrid breeding in these crop areas.

(vii) *Better plant types*

In future, the hybrid breeding efforts, will receive greater emphasis for drastic changes of the plant types of the various crops. This would be primarily for an increase in their fertilizer responsiveness, inherent potentialities for higher yield, nonlodging behaviour, right type of maturity and with a builtin potential to react to harsh environments or a combination of these in a single plant type. The job of accumulating genes for all these characters is a stupendous one, however, all is needed in a productive cultivar and weakness of one particular trait may become limiting to higher production. Presently the usage of hybrid varieties in a number of the agricultural areas are drastically reduced by the brief but severe stresses caused by these. Even a different maturity behaviour of a crop in an otherwise potential area may not find a place in local practices of crop rotations and may altogether throw a hybrid out of gear. An instance is that alongwith good yield and adaptability, the hybrid sorghum CSH-1 finds much favour with the farmers in Maharashtra in India while the late maturity of the high yielding maize hybrids disqualifies it for its large scale cultivation in Uttar Pradesh where the farmers generally prefer the high yielding hybrids with parallel maturity and quality to that of the local varieties.

It is now well recognized that for increased physiological and biochemical efficiencies of the plants and for further lifting the yield barrier to a high level, the plant morphology and physiology has to be changed drastically. Production of high yields by the hybrids would need high absorption and assimilation of balanced nutrients in high and higher doses. Incorporation of dwarfing genes alongwith other desirable agronomical characters in cereal crops like wheat, rice as well as in sorghum has paid rich dividends. This concept is further being extended to maize and bajra programmes. Promising results have been obtained by Dr. Leng by incorporating br2 gene into the standard inbred lines and the hybrids. This has attracted the attention of many maize breeders and the future may see development of dwarf hybrids in maize.

The search for the genes governing thermo and photononsensitivity is another very desirable aspect that will receive much attention in future. The incorporation of such genes in rice and wheat varieties have facilitated the production of possibly two crops where only one grew before. Such varieties may also be used over a wide range of ecological conditions and perhaps, beyond the frontiers of national boundaries.

Prolificacy has been observed to be a desirable attribute in maize breeding programmes. This is, especially, true when the maize is to be grown in high plant population densities and the need to be better adapted to the adverse conditions of drought, low soil fertility etc. with a lower tendency for barren plants. Presently plants with large single ears are preferred. However, in future to boost the production still further prolificacy may be an important aspect of the maize breeding programmes.

(viii) *Cooperative research and heterosis breeding*

The trend in recent years in crop improvement programmes is to develop crop varieties with very high level of production. It has been felt that a well knitted, multidisciplinary, integrated, cooperative research effort in which a team of reasearch workers pool their resources, knowhow and effort; accomplishes the job efficiently. Isolated and individual research work though effective in small measure sometimes produces aimless excellence. It could be better harnessed and meaningfully utilized in a cooperative research programme. The advantages of such cooperative research in evolving widely adapted hybrid varieties, are obvious and many. Some of the colossal jobs in hybrid breeding programmes may not be handled individually and need to be tackled in a team effort. Breeding for diseases, insects, biochemical attributes etc. could be better done by plant breeders in cooperation with plant pathologist, entomologist, biochemist, statistician etc. The plant breeders though concerned with over all aspects of breeding cannot efficiently accomplish their objectives unless they obtain the effective cooperation from the scientists in the related disciplines. In India the average level of yield improvement in wheat, rice and sorghum in recent years could be safely attributed to the team effort and the active cooperation of a brand of scientist from a number of disciplines and a purposeful cooperation between the agencies like I.C.A.R., Rockefeller Foundation, U S.A.I.D. and the Experiment Station of the various state departments. Inspired by this successful experiment recently the coordinated research progromme has been established in almost all economic crop species in India. In future, cooperative crop improvement programmes would see rather a much more expanded participation of scientists belonging to disciplines like agricultural engineering, economics, food nutrition, physiological and biochemical genetics and even the research administration. In nutshell, there is no effective alternative to cooperative team effort in solving the complex problems of production, to maintain the tempo of progress and to bring in new innovations in the hybrid breeding programmes.

# GLOSSARY

# GLOSSARY

**Adaptation :** Adjustment of an organism to a new environment.

**A-line :** The male sterile line usually used as seed parent in hybrid seed production programmes as in sorghum, bajra or maize.

**Allele :** One of a pair or series of alternative contrasting form of a gene that is located at given locus in homologous chromosomes. Alleles are symbolized with the same basic symbol (T for tall peas and t for dwarf).

**Aneuploid :** A cell or an organism containing chromosomal complements different from the normal n from gametes and 2n from somatic cells example nullisomic (2n-2), monosomic (2n-1), trisomics (2n+1) etc.

**Antibody :** An organic substance which acts in antagonism to a foreign substance.

**Antigen :** An organic substance (usually a protein) which stimulates the production of antibodies when introduced into a living cell.

**Asexual reproduction :** The system of reproduction which does not involve fusion of male and female gametes.

**ATP :** Adenosine triphosphate - an energy rich compound participating in energy storage and energy using reactions in cells.

**Auxotroph :** A mutant (usually in neurospora or bacteria) which requires an additional supply of certain growth factor(s) in the minimal medium for its growth.

**Backcross :** The cross of $F_1$ hybrid with one of the parents.

**Backcross method of breeding :** It is a method of breeding in which the desirable character(s) of a non-recurrent (donor) parent are added to the genetic background of recurrent (recipient) parent through subsequent generations of backcrossing and selection.

**Balanced heterosis :** It is a type of true heterosis which occurs from the balanced combinations of genes in a hybrid.

**Balanced polymorphism :** The condition in which two or more types of individuals or genes are maintained in the same breeding population.

**Biometrics :** The science dealing with the application of statistical methods to biological problems.

**Biosystematics :** The science dealing with the study and analysis of living

relations of the organisms (with respect to their origin evolution and diversification etc ).

**Biparental progenies (of a cross) :** The third generation of a cross obtained by the random mating of $F_2$ individuals taken in pair.

**B-line :** The fertile counterpart of a line. This line does not have fertility restorer genes and is used as the male parent to maintain the A-line.

**Breeding :** The art and science of changing plants or the animals genetically.

**Clone :** A group of individuals derived by a single original plant propogated by vegetative means.

**Codominant genes :** Alleles, each of which produce an independent effect in $F_1$.

**Coefficient of inbreeding :** A quantitative measure of the extent of inbreeding of an organism or population. It is usually denoted by F.

**Combinational heterosis :** The heterosis in quantitative characters resulting from the overall combination of the favourable cumulative effects of a number of componental characters.

**Combining ability :** General combining ability : The comparative ability of a line or a genetic stock to combine with a tester or a group of testers. Specific combining ability : The deviation in the performance of a specific single cross from the performance expected on the basis of general combining ability.

**Complementary genes :** Those genes which have more or less similar phenotypic expression individually but when they come together they interact to produce a new character expression. If two such genes are complementary for a do minant effect, a 9 : 7 ratio results in $F_2$; if two are complementary for a recessive effect, a 15 : 1 ratio results in $F_2$.

**Composites :** The advanced generations seed mixture of an intervarietal or interracial cross.

**Convergent improvement :** A system of double back crossing for the purpose of improving each of the two inbred lines without greatly modifying the yield of their $F_1$ cross.

**Correlation coefficient :** A statistical constant which measures the degree of mutual association between two or more series of variables. It is usually represented by "r".

**Cross :** The product of the mating between two or more parents of dissimilar genetic constitution. The various types of crosses utilized in heterosis breeding programmes are single, threeway, double crosses, topcross, multiple crosses etc.

**Cross pollination :** Transfer of pollen from the anther of one plant to the stigma of another plant. It effects the union of genetically dissimilar gametes.

**Cross pollinated crops :** An assembly of genetically heterogygous individuals under commercial cultivation which share a common gene pool and in which each individual partakes new genetype generation after generation.

**Cultivars :** Usually refers to the cultivated varieties.

**Cytoplasmic :** Concerned with the cytoplasm in a cell.

**Cytoplasmic male sterility :** A type of pollen sterility which is transmitted through the cytoplasm. Its inheritence is maternal.

**Detasseling :** The process of mechanical removal of tassels from the plants. This is extensively practiced in the hybrid seed production of maize to facilitate large scale controlled crossing.

**Diallele cross :** A diallele cross can be defined as all possible combinations *i.e.*, n(n-1)/2 of single crosses among n parents.

**Dichogamy :** The maturing of male and female gametes at different times. When the male gametes matures earlier than the female it is called protandry, but when female matures first, it is called protogyny.

**Dioecious :** The condition in which male and female sexes are produced on different individuals.

**Dominance :** The phenomenon in which the dominant gene has an over-riding effect on its allele in such a way that the heterozygote (Aa) is phenotypically indistinguishable from the dominant homozygote (AA)

**Dominant gene :** A gene which expresses itself in the heterozygote condition and overrides the effect of its contrasting (recessive) allele.

**Double cross hybrid :** A hybrid obtained by crossing two single crosses *i.e.*, $(A \times B) \times (C \times D)$.

**Double topcross :** A cross obtained from crossing a single cross with an open pollinated variety.

**Drought resistance :** The capacity of the plant to survive and develop in drought or dearth of water with little or no injury.

**Emasculation :** Artificial removal of the anthers from the bud or the flower before the anther is mature so as to prevent its own pollination.

**Embryo :** The portion of seed which contains the dormant, miniature, rudimentary plant. It arises from the zygote.

**Endosperm :** The nutritive tissue formed inside the embryo sac in the seed. It arises from the triple fusion of a sperm nuclei with the polar nuclei of embryo sac.

**Enzyme :** A complex organic substance (a specific protein wholly or in parts) that accelerates, regulates and catalyses a specific biochemical reaction in living organism. Genes produce their effects by the activation or inactivation of specific enzyme.

**Epistasis :** The phenomenon in which a non-allelic gene or gene combination exert a dominant effect over another gene or combination of genes (non-allelic interaction).

**Evolution :** The process of the origin of the organisms (Varieties, species, genera, families etc.).

**Fertility :** The ability of an organism to form viable off springs.

**Fertility restorer gene :** Usually a gene which when put into a cytoplasmic male sterile background is able to bring back the production of normal functional pollen grains.

**Fitness :** The relative ability of genotype to produce viable offsprings in the next generation. It is a measure of reproductiveness.

**Gamete :** A matured sex cell, capable of fusing with another to form a zygote.

**Gamete selection :** It is a type of selection for detecting and combining of desirable gamete from a genetically variable heterozygous population into the background of an inbred line of known performance and combining ability.

**Gametocides :** Chemicals which check or retard the formation of gametes.

**Geitonogamy :** A type of self pollination in which the pollen is obtained from another flower of the same plant.

**Gene :** A hypothetical unit situated on a fixed chromosomal locus which is responsible for the expression and transmission of a heritable trait.

**Gene frequency :** Proportion of a gene or allele or its series present in a population or a sample thereof. It is usually expressed as number between 0 to 1.

**Gene Interaction :** The modification of gene action by a nonallelic gene or genes.

**Gene pool :** Sum total of all genes in a breeding population.

**Genetic diversity :** The genetic differences as observed between individuals, or genetic stocks with respect to individual trait or an array of traits.

**Genetic equilibrium :** A state in which the frequency of alleles of a given gene tends to remain constant in an interbreeding organism through succeeding generations.

**Genetic homeostasis :** The ability of a random mating population to equilibrate its genetic composition so as to resist sudden environmental changes.

**Genetic parameters :** Statistical constants representing the population. Example standard deviation. Statistics is the estimate of parameter made from a sample. Example standard error.

**Genome** : A complete set of chromosomes (hence of genes) inherited as a unit of one parent of a particular diploid species.

**Genome analysis** : The process of determining the genomic relationships between the individuals, species, or other populations.

**Genotype x environment interaction** : The interplay in effect of the genetic and non genetic factors on the development of an organism.

**Geographical diversity** : The diversity of the biological population produced by the presence of a geographical barrier such as mountains, rivers, canyons etc.

**Germplasm** : In plant breeding sense, germplasm is the sum total of genetic stocks of a particular crop species.

**Germplasm complexes** : The advanced generation of mixed seeds obtained by a purposeful intermixing of a number of genotypes or hybrids among them.

**Heterobeltiosis** : Heterosis expressed over the better parent of the cross.

**Heterocaryon** : A cell of a mycelium containing two or more genetically different nuclei pair or which do not pair or fuse but divide independently or an organism consisting of such cells.

**Heterocaryotic vigour of fungi** : The hybrid vigour brought about by two different nuclei in a heterocaryon in a single cell of a mycelium.

**Heterofertilization** : The process in which egg and polar nuclei are fertilized by different sperm nuclei obtained by more than one pollen tubes.

**Heterozygosity** : The phenomenon in which the homologous chromosomes of an organism possess different genes of the same allelic series.

**Heterozygote** : An organism with one or more heterozygous pairs of genes or unlike alleles at one or more corresponding loci. As a result of heterozygosity, the organism will not breed true.

**Homozygote** : An organism with identical genes at corresponding loci on homologous chromosomes.

**Hybrid** : The individual obtained by fusion of two genetically dissimilar gametes.

**Hybridization** : The process of making hybrids.

**Hybrid vigour** : The increased vigour obtained by crossing two dissimilar individuals.

**Heterosis** : The phenomenon in which the crossing of two dissimilar individuals ($F_1$), shows an increased or decreased vigour over the mean of the parents or over the better parent.

**Inbred line** : A practically uniform, genetically homozygous line produced by inbreeding and selection.

**Inbreeding :** The mating of closely related organisms.

**Inbreeding depression :** The loss of vigour as a consequence of inbreeding. It is primarily due to the break down of specific gene system governing the expression of vigour governing a particular trait or traits and is often accompanied by reduction in yield, size, fecundity etc.

**Introgressive hybridization :** A type of hybridization in which a number of genes or gene block of one species are added to the genetic background of another species by crossing and often by backcrossing.

**Kernel :** The term refers to the whole grain in the cereal crops like maize, rice, wheat etc.

**Line :** This term usually refers to a group of individuals obtained from a common ancestry. In maize programme, it is synonym to inbred line.

**Line breeding :** Mating of selected members of a line among themselves in successive generation to fix desirable characteristics.

**Line X Tester analysis :** A system of testing lines for combining ability in the genetic background of a number of proven testers.

**Luxuriance :** It refers to the phenomenon in which the crossing of two parental forms brings in an excessive, accidental, unadaptable and often unbalanced expression of an attribute.

**Male sterility :** An inherited physiological abnormality in which the male gamete is rendered functionless.

**Mating systems :** The system in which individuals are arranged in pairs leading to sexual reproduction. There are following system of matings :

*Random Mating* : Arrangements of pairs is by chance *i.e.* each individual has equal chances to mate with another. Example cross pollinated crops.

*Inbreeding* : Mating of closely related individuals, example-self pollinated crops.

*Phenotypic assortative mating* : Mating of individuals with similar phenotypes-example crossing between tall individuals with AA × AA or Aa or Aa × AA and Aa × Aa genotypes.

*Phenotypic disassortative mating* : Mating of individuals with contrasting phenotypic characters. Example-crossing between tall × dwarf plants. (Allard, 1960).

**Mitochondria :** Small cytoplasmic organelles in which cellular respiration takes place and the usable cell energy is liberated. These are actually the small power houses of the cell.

**Modifying gene :** The gene that affects, retards or diminishes the phenotypic expression of another non allelic gene.

**Monoecious** : The condition in which both male and female sex organs are produced separately but on the same plant.

**Monoploid** : An organism having a single genome or set of chromosomes. Haploids of diploid species are monoploids but haploids of polyploids are not monoploids since they contain more than one genome.

**Multiline varieties** : The phenotypically similar variety having a purposeful mixture of genetically distinct desirable lines conferring resistance to diseases, pests etc. The mixture gives more adaptability to the variety to be grown under adverse conditions of crop production.

**Multivariate analysis** : The statistical analysis obtaining the information about the genetic divergences between the various populations based on information obtained from a number of characters.

**Mutagens** : Chemicals which are generally used for the artificial induction of mutations. Example EMS (Ethyl Methane Sulphonate).

**Mutation** : The sudden heritable change in an organism which does not arise as a consequence of recombination on segregation.

**Mutational heterosis** : It is a type of true heterosis which results from the occurrence of the balanced types of mutations in plants.

**Natural selection** : A type of selection which operates automatically in nature. In this the fittest survives and the rest are wiped out.

**Non recurrent parent** : The donor parent in the backcrossing program. The desirable character of this parent is added in the genetic background of recurrent parent.

**Outbreeding** : Mating of unrelated individuals.

**Outcross** : A type of natural cross obtained from the crosses of a number of unknown genotypes.

**Overdominance** : An effect of the heterozygote (Aa) which is greater than the effect of homozygous dominant (AA).

**Ovule** : The term generally applied to the whole seed forming apparatus inside an ovary *i.e.*, nucellus plus the integument.

**Panmictic population** : The population obtained as a consequence of complete random mating.

**Parthenocarpy** : The development of fruits without fertilization and the formation of normal seeds. Example Banana.

**Parthenogenesis** : The development of an individual from the female gamete without fertilization.

**Phenotype** : The visible manifestation of the genotype produced as a consequence of growth and development.

**Phenotypic stability** : The stability of a genotype (or genotypes) over a

spectrum of environmental conditions. (also known as developmental homeostasis).

**Plant introduction :** Refers to the method of breeding in which the breeders obtain useful genetic stocks from outside his own establishment to utilize it in his selection or hybridization programmes.

**Pollination :** The process by which the pollen is transferred from the anthers to the stigma.

**Polyallele crossing :** Crossing of several inbred lines with each other to determine their respective combining ability.

**Polycross :** The hybrid progenies obtained by crossing with a large number of selected pollen parents in insolation under natural conditions.

**Polycross testing :** It is a system of testing by growing a number of individuals or lines in isolation and open pollination following which the progenies of each may be grown separately to evaluate the general combining ability of their parent.

**Polymorphism :** Presence of more than one allele or distinct form in a population.

**Polyploidy :** The phenomenon of increase in number of chromosomes in multiples of the haploid number. Example 3n, 4n, 6n, etc.

**Population :** Statistically, it is an assembly of a large series of infinite individuals. Genetically it is a community of individuals which shares a common gene pool.

**Pseudo-dominance :** The phenomenon of the apparent dominance of a recessive gene in the area opposite a chromosome deficiency.

**Pseudo-heterosis :** See luxuriance.

**Pure line :** A completely homozygous mating group obtained by successive self pollination.

**Qualitative characters :** The characters which show descrete variation and easily identified by visual observations. Examples, flower colour, fruit shapes etc.

**Quantitative characters :** The characters which show continuous variation such that visual identification of individual genes segregation are not possible. These are usually governed by the cumulative effect of polygenes and are highly influenced by environmental conditions. Examples are yield per plant, fruits per plant etc.

**Random :** Derived by chance without bias or descrimination.

**Recessive gene :** The gene which is not able to express itself in the presence of a dominant gene in the heterozygote condition.

**Reciprocal hybrids** : Two hybrids produced by crossing the same parents but the male of first is used as female in the another and similarly the female of first is used as male in another such as (A × B) and (B × A).

**Relative heterosis** : Usually refers to the heterosis expressed over the mid parental value of a cross.

**Rouging** : The act of removing undesirable individuals from varietal mixtures or seed production plots.

**Seed** : The matured ovule having all the essential structures to produce a new plant.

**Breeders seed** : The seed material directly produced by the breeder which provide the source for the initial and recurring increase of foundation stock.

**Foundation seed** : The seed stocks that are so handed as to most nearly maintain the specific genetic identity and purity of the original stock and provide the source for the production of all certified and registered seed.

**Registered seed** : The progeny of foundation seed that is so handled as to most nearly maintain the satisfactory genetic purity and has been approved by a certifying agency.

**Certified seed** : The progeny of a foundation or registered seed which maintains the satisfactory genetic identity and purity and has been certified and approved by the certifying agency.

**Seed parent** : The female (pistillate) parent of a hybrid.

**Selection** : The process in which a number of individuals with certain desirable characteristics are favoured for further reproduction.

**Selective male gametocides** : The chemicals which selectively kill the pollen grains of a plant without affecting the viability of the egg cells. Example FW 450.

**Selfing** : The process of putting the pollen of the same flower on its stigma *i.e.*, enforcing self pollination. It is extensively practiced in maize and other cross pollinated crops to obtain inbred lines for utilization in the hybrid programme.

**Self fertile** : Refers to an organism which is capable of fertilization and setting seed.

**Self incompatibility** : The inability of a functional gamete or the plant to set seed when self pollinated, even though it forms normal zygotes when cross pollinated.

**Self pollination** : The transfer of the pollen from the anther to the stigma of the same flower or the plant. It is also the union of genetically similar gametes.

**Self pollinated crops** : An assembly of homozygous plants. These crops often have one single genotype and reproduce it as such from generation to generation.

**Self sterile** : The organism which fails to fertilize and set seeds after self pollination.

**Semi-sterility** : A state of only partial fertility in plant zygotes usually associated with chromosomal translocations.

**Sex reversal** : A change in the characteristics of an organism from female to male and vice versa.

**Sib mating** : Sibbing or crossing at random the two or more individual obtained from the same parentage. It is a form of inbreeding and refers to brother-sister mating.

**Single cross** : A cross between two genetically dissimilar parents.

**Somatopalstic sterility** : A type of sterility which results as a consequence of the collapse of fertilized ovules or zygote during the embryonic or early developmental stages due to disturbance in embryoendosperm relations.

**Species** : A unit of taxonomic classification containing group of individuals enough alike so that it may be reasonably assumed that they have arisen from a common ancestor.

**Standard heterosis** : Heterosis expressed over the standard or check variety. It has also been referred as 'useful heterosis'.

**Step allelism** : The concept of series of alleles with graded effects on the same character.

**Sterility** : The phenomenon in which an organism is infertile and is incapable of reproducing.

**Strain** : The mating group within a variety or species with distinct morphological or physiological features.

**Symbols** : In breeding programmes, the following symbols are generally used. They cannote the following meaning :—

$F_1, F_2, F_3$...Filial generations of a cross.
$BC_1$, $BC_2$, $BC_3$...Backcrossed generations of a cross.
$S_1$, $S_2$, $S_3$...Selfed generations from an ancestral ($S_0$) plant.
$I_1$, $I_2$, $I_3$...Inbred generations.
$C_1$, $C_2$, $C_3$...Generations produced after colchicine treatment.
$M_1$, $M_2$, $M_3$...Generations produced after treatment with a mutagen.
$Syn_1$, $Syn_2$, $Syn_3$ ..Generations of synthetic varieties.
$P_1$, $P_2$, $P_3$...Generations of a parental population.

**Synthetic varieties** : These are the open pollinated advanced generation population of a number of hybrids obtained by crossing a number of tested lines growen in isolation.

**Testcross :** The cross of the $F_1$ with the recessive homozygous parent *i.e.*, Aa × aa.

**Threeway cross :** The cross between a single cross and a inbred line. With 3 lines (A,B,C), it could be represented as (A×B)×C. It has been generally used in the Maize Breeding Programmes for the production of commercial sweet maize hybrids.

**Topcross :** The inbred × Variety cross. It is generally used for screening inbred lines for general combining ability.

**Transgressive segregation :** The appearance of individuals showing an extreme development of a character than either of the parent in $F_2$ or later generations. This usually occurs because of cumulative and complementary effect of genes contributed by the parents of the cross.

**Trihybrid :** Hybrid derived from a cross between parents which differ with respect to three specified genes.

**Triple cross :** The cross between two threeway crosses. With 6 lines (A,B, C,D,E,F) it could be represented as ( (A×B)×C)×(D×E)×F).

**Useful heterosis :** See standard heterosis.

**Variance :** *Additive genetic variance* : The genetic variability of a quantitative trait in a population that is due to additive gene action.

*Dominance genetic variance* : The genetic variability of a quantitative trait in a population that is due to dominance deviation.

*Epistatic variance* : The genetic variability of a quantitative trait in a population that is due to non allelic gene interaction.

**Variation :** Differences amongst the individuals arising as a consequence of differences in the genetic make up; the effect of the environment or an interplay of both. It is the chief characteristics of living organisms and is the basis of evolution.

**Variety :** An agricultural variety is a population of similar individuals having common identifiable plant, fruit or seed characteristics alongwith a good agronomic base This is generally utilized for commercial cultivation.

**Viability :** It relates to the capability to live and develop normally.

**Wide cross :** The type of cross in which the parental populations differ greatly in their genetic constitution.

**Xenia :** The immediate effect of pollen (*i.e.*, male gamete) on the endosperm. It is frequently observed in open pollinated maize with respect to kernel colour.

**Zygote :** The cell formed by the fusion of male and female gametes.